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(54) Title: HUMAN INTERFERON TAU COMPOSITIONS AND METHODS OF USE			
(57) Abstract			
<p>The present invention describes the isolation and characterization of multiple forms of human interferon-τ. Protein and nucleic acid coding sequences for the multiple forms are disclosed. In further aspects, the invention relates to methods of producing and using human interferon-τ molecules.</p>			

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HUMAN INTERFERON TAU COMPOSITIONS AND METHODS OF USE

Field of the Invention

The present invention relates to human interferon- τ compositions and methods of use.

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15 **Background of the Invention**

Conceptus membranes, or trophoblast, of various mammals produce biochemical signals that allow for the establishment and maintenance of pregnancy (Bazer, *et al.*, 1983). One such protein, ovine trophoblast protein-one (oTP-1), was identified as a low molecular weight protein secreted by sheep conceptuses between days 10 and 21 of pregnancy (Wilson, 20 *et al.*; Bazer, *et al.*, 1986). The protein oTP-1 was shown to inhibit uterine secretion of prostaglandin F₂-alpha, which causes the corpus luteum on the ovary to undergo physiological and endocrinological demise in nonpregnant sheep (Bazer, *et al.*, 1986). Accordingly, oTP-1 has antiluteolytic biological activity. The primary role of oTP-1 was assumed to be associated with the establishment of pregnancy.

25 oTP-1 was subsequently found to (i) exhibit limited homology (50-70%) with interferon alphas (IFN α) of various species (Imakawa, *et al.*), and (ii) bind to a Type I interferon receptor (Stewart, *et al.*). Despite some similarities with IFN α , oTP-1 has several features that distinguish it from IFN α including the following: oTP-1's role in reproductive biochemistry (other interferons are not known to have any role in the biochemical regulation of reproductive 30 cycles), oTP-1's cellular source — trophoblast cells (IFN α is derived from lymphocytes cells), oTP-1's size — 172 amino acids (IFN α is typically about 166 amino acids), and oTP-1 is weakly inducible by viruses (IFN α is highly inducible by viruses). The International Interferon Society recognizes oTP-1 as belonging to an entirely new class of interferons which have been named interferon-tau (IFN τ). The Greek letter τ stands for trophoblast.

The interferons have been classified into two distinct groups: type I interferons, including IFN α , IFN β , and IFN ω (also known as IFN α II); and type II interferons, represented by IFN γ (reviewed by DeMaeyer, *et al.*). In humans, it is estimated that there are at least 17 IFN α non-allelic genes, at least about 2 or 3 IFN β non-allelic genes, and a single IFN γ gene.

5 IFN α 's has been shown to inhibit various types of cellular proliferation. IFN α 's are especially useful against hematologic malignancies such as hairy-cell leukemia (Quesada, *et al.*). Further, these proteins have also shown activity against multiple myeloma, chronic lymphocytic leukemia, low-grade lymphoma, Kaposi's sarcoma, chronic myelogenous leukemia, renal-cell carcinoma, urinary bladder tumors and ovarian cancers (Bonnem, *et al.*;
10 Oldham). The role of interferons and interferon receptors in the pathogenesis of certain autoimmune and inflammatory diseases has also been investigated (Benoit, *et al.*).

IFN α 's are also useful against various types of viral infections (Finter, *et al.*). Alpha interferons have shown activity against human papillomavirus infection, Hepatitis B, and Hepatitis C infections (Finter, *et al.*; Kashima, *et al.*; Dusheiko, *et al.*; Davis, *et al.*).

15 Significantly, however, the usefulness of IFN α 's has been limited by their toxicity: use of interferons in the treatment of cancer and viral disease has resulted in serious side effects, such as fever, chills, anorexia, weight loss, and fatigue (Pontzer, *et al.*, 1988; Oldham). These side effects often require (i) the interferon dosage to be reduced to levels that limit the effectiveness of treatment, or (ii) the removal of the patient from treatment. Such
20 toxicity has reduced the usefulness of these potent antiviral and antiproliferative proteins in the treatment of debilitating human and animal diseases.

A recent PCT International Application (WO 94/10313, published 11 May 1994) described the production of interferon- τ proteins. The antiviral and anticellular proliferation properties of these proteins and polypeptides were also described. The disclosure discussed
25 ovine interferon- τ and an isolate of human interferon- τ .

Summary of the Invention

The present invention relates to compositions of and methods employing human interferon- τ 's. In one embodiment, the invention includes an isolated nucleic acid molecule that
30 encodes a human interferon- τ . Several variants of human interferon- τ (HuIFN τ) are disclosed herein, including HuIFN τ 1, HuIFN τ 2, HuIFN τ 3, HuIFN τ 4, HuIFN τ 5, HuIFN τ 6 and HuIFN τ 7. The nucleic acid molecules of the present invention include nucleic acid molecules having the following sequences: SEQ ID NO:29, SEQ ID NO:15, SEQ ID NO:11, SEQ ID NO:19, SEQ ID NO:13, SEQ ID NO:7 and SEQ ID NO:9.

The nucleic acids of the present invention may further include sequences encoding leader sequences for the human interferon- γ which they encode, for example, SEQ ID NO:27 or SEQ ID NO:28.

The nucleic acid molecules described above encode human interferon- γ proteins and polypeptides of the present invention. The amino acid sequence of a human interferon- γ protein is typically a variant sequence of the human interferon- γ core sequence (HuIFN γ CS; SEQ ID NO:34). It will be understood, however, that variants of the core sequence can be modified by introducing conservative amino acid substitutions that do not significantly change the activity or characteristics of the modified variant with respect to the corresponding unmodified variant. Such modified variants are also included within the scope of the present invention. In a preferred embodiment, such modified variants of the present invention do not include modifications at positions 84 and 85 of a mature interferon- γ amino acid sequence.

In one general embodiment the HuIFN γ proteins or polypeptides are "Group I" proteins or polypeptides, derived from the core sequence represented as SEQ ID NO:31. Examples include HuIFN γ 1 (SEQ ID NO:30) and HuIFN γ 2 (SEQ ID NO:16). In another general embodiment they are "Group II" proteins or polypeptides, derived from the core sequence represented as SEQ ID NO:32 and exemplified by HuIFN γ 3 (SEQ ID NO:20), HuIFN γ 4 (SEQ ID NO:12) and HuIFN γ 5 (SEQ ID NO:14). In still another general embodiment they are "Group III" proteins or polypeptides, derived from the core sequence represented as SEQ ID NO:33, with examples including HuIFN γ 6 (SEQ ID NO:8) and HuIFN γ 7 (SEQ ID NO:10).

A second aspect of the invention includes an expression vector having a nucleic acid containing an open reading frame that encodes a human interferon- γ , including the nucleic acid and polypeptide sequences described above. The vector further includes regulatory sequences effective to express said open reading frame in a host cell. The regulatory sequence may include sequences useful for targeting or secretion of the human IFN γ polypeptide: such sequences may be endogenous (such as the normally occurring human IFN γ leader sequences, present, for example, in SEQ ID NO:27) or heterologous (such as a secretory signal recognized in yeast, mammalian cell, insect cell, tissue culture or bacterial expression systems). In the expression vector, regulatory sequences may also include, 5' to said nucleic acid sequence, a promoter region and an ATG start codon in-frame with the human interferon- γ coding sequence, and 3' to said coding sequence, a translation termination signal followed by a transcription termination signal.

In a further embodiment, the invention includes a method of recombinantly producing human interferon- γ . In the method, the expression vector, containing sequences encoding a

human interferon- τ open reading frame (ORF), is introduced into suitable host cells, where the vector is designed to express the ORF in the host cells. The transformed host cells are then cultured under conditions that result in the expression of the ORF sequence. Numerous vectors and their corresponding hosts are useful in the practice of this method of the invention, including, lambda gt11 phage vector and *E. coli* cells. Other host cells include, yeast, mammalian cell, insect cell, tissue culture, plant cell culture, transgenic plants or bacterial expression systems.

In another embodiment, the invention includes an isolated human interferon- τ protein or polypeptide. The protein may be recombinantly produced. Further, the protein or polypeptide may include any of the following human interferon- τ sequences: SEQ ID NO:30, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:8, and SEQ ID NO:10.

The invention further includes a method of inhibiting tumor cell growth. In the method, the tumor cells are contacted with a human τ -interferon polypeptide at a concentration effective to inhibit growth of the tumor cells. The human interferon- τ may be a part of any acceptable pharmacological formulation. Tumor cells whose growth may be inhibited by human interferon- τ include, but are not limited to, human carcinoma cells, hematopoietic cancer cells, human leukemia cells, human lymphoma cells, and human melanoma cells. In one embodiment, the tumor cells are steroid-sensitive tumor cells, for example, mammary tumor cells.

In yet another embodiment of the present invention, human interferon- τ polypeptides are used in a method of inhibiting viral replication. In this method, cells infected with a virus are contacted with human τ -interferon at a concentration effective to inhibit viral replication within said cells. The human interferon- τ may be a part of any acceptable pharmacological formulation. The replication of both RNA and DNA viruses may be inhibited by human interferon- τ polypeptides. Exemplary RNA viruses include human immunodeficiency virus (HIV) or hepatitis c virus (HCV). An exemplary DNA virus is hepatitis B virus (HBV).

In yet another aspect, the present invention includes a method of enhancing fertility in a female mammal. In this method, an effective mammalian fertility enhancing amount of human interferon- τ is administered to the female mammal in a pharmaceutically acceptable carrier.

The invention also includes isolated human interferon- τ polypeptides. These polypeptides are derived from the human interferon- τ amino acid coding sequence and are

typically between about 20 and 172 amino acids in length, preferably between about 85 and 172 amino acids in length.

Also included in the invention is a fusion polypeptide that contains a human interferon- τ polypeptide that is between 8 and 172 amino acids long and derived from a human interferon- τ amino acid coding sequence, and a second soluble polypeptide.

In one embodiment, human interferon- τ sequences are used in hybrid fusion constructs with other type I interferons to reduce the toxicity of the other type I interferons (*e.g.*, interferon- α and interferon- β). Experiments performed in support of the present invention suggests that the portion of an alpha or beta interferon molecule responsible for the increased cytotoxicity of those molecules with respect to that of interferon tau resides in the first about 8 - 37 amino acids. Accordingly, the DNA encoding such a reduced-cytotoxicity hybrid molecule is formed of a 5' end segment that encodes the N-terminal amino acid sequence of an interferon-tau polypeptide, and a 3' end segment that encodes the C-terminal amino acid sequence of a non-tau interferon type I polypeptide. The two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about amino acid residues 8 and 37. In one embodiment, the 5' end segment further includes a leader sequence. Such hybrid construct may be used to confer, *e.g.*, the antiviral activity of an IFN α with the reduced cytotoxicity of an IFN τ . In a broader context, such compositions may be used to reduce the toxicity of the other types of interferons when the interferons are used in pharmaceutical formulations or in therapeutic applications.

Examples of sequences encoded by the 5' end segment include variants derived from the first 8 to 37 residues of SEQ ID NO:34, such as the first 8 to 37 residues of a sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:20, and SEQ ID NO:30.

The 3' end segment may encode, in various embodiments, an amino acid sequence derived from an interferon alpha 1, alpha 2, beta or omega. The 3' end segment may also encode a consensus sequence from any of the above. Preferred embodiments are where the sequence is derived from a human source, such as a human IFN α or human IFN β . An exemplary human IFN α sequence (HUMIFNN) suitable for the construction of a reduced-toxicity hybrid molecule can be obtained from GenBank under accession number M28585.

In a general embodiment, the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 28. In another general embodiment, the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 22. In yet another general

embodiment, the two segments are spliced in a region corresponding to the portion of a mature interferon polypeptide between about residues 8 and 16.

The invention also includes purified antibodies that are immunoreactive with human interferon- τ . The antibodies may be polyclonal or monoclonal.

5

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

10 **Brief Description of the Figures**

Figure 1 presents the complete nucleic acid and amino acid sequence of an OvIFN τ sequence.

Figure 2 shows a comparison of the predicted protein sequences of a human interferon- τ gene and an ovine interferon- τ gene. Divergent amino acids are indicated by presentation of
15 the alternative amino acid on the line below the nucleic acid sequences.

Figures 3A and 3B present an alignment of nucleic acid sequences encoding IFN τ polypeptides.

Figures 4A and 4B present an alignment of amino acid sequences of IFN τ polypeptides.

20 **Detailed Description of the Invention**

I. Definitions

Human Interferon- τ (HuIFN τ) refers to any one of a family of interferon proteins that contains (i) a variant sequence of the human interferon- τ core sequence (HuIFN τ CS; SEQ ID NO:34), or (ii) a sequence corresponding to a particular variant of HuIFN τ CS (SEQ ID NO:34)
25 and containing conservative amino acid substitutions, where the substitutions do not significantly change the activity or characteristics of that particular variant. Examples of HuIFN τ variant amino acid sequences include HuIFN τ sequences represented herein by HuIFN τ 1 (SEQ ID NO:30), HuIFN τ 2 (SEQ ID NO:16), HuIFN τ 3 (SEQ ID NO:20), HuIFN τ 4 (SEQ ID NO:12), HuIFN τ 5 (SEQ ID NO:14), HuIFN τ 6 (SEQ ID NO:8) and HuIFN τ 7 (SEQ
30 ID NO:10). Activities and characteristics associated with HuIFN τ include at least one, preferably two or more, from the following group of characteristics: (a) expressed during embryonic development stages, (b) anti-luteolytic properties, (c) anti-viral properties, and (d) anti-cellular proliferation properties.

A *human interferon- τ polypeptide* is a polypeptide having between about 20 and 172 amino acids, preferably between about 85 and 172 amino acids, derived from a mature human interferon- τ amino acid coding sequence, where said 20 to 172 or 85 to 172 amino acids are contiguous in native interferon- τ . Such contiguous amino acid regions can also be assembled
5 into polypeptides where two or more such interferon- τ regions are joined that are normally discontinuous in the native protein.

A *mature interferon- τ polypeptide* is a full-length interferon- τ polypeptide less the leader sequence. A mature human interferon- τ polypeptide typically contains between about 171 and 172 amino acid residues.

10 A *core sequence* is sequence having at least one variable position represented by two or more individual amino acid residues. In an amino acid core sequence, such a variable position is indicated as "Xaa". Each core sequence defines two or more *variant sequences*, or *variants*, having a single amino acid residue at each position. For example, the core sequence "Ser Xaa Phe", where Xaa is Leu or Ile, defines the variant sequences "Ser Leu Phe" and "Ser
15 Ile Phe".

Conservative amino acid substitutions are substitutions which do not result in a significant change in the activity (*e.g.*, antiviral activity) or tertiary structure of a selected polypeptide. Such substitutions typically involve replacing a selected amino acid residue with a different residue having similar physico-chemical properties. For example, substitution of
20 Glu for Asp is considered a conservative substitution since both are similarly-sized negatively-charged amino acids. Groupings of amino acids by physico-chemical properties are known to those of skill in the art and can be found, for example, in Schulz and Schirmer (1979).

When a first polypeptide fragment is said to *correspond* to a second polypeptide fragment, it means that the fragments or regions thereof are essentially co-extensive with one
25 another when the sequences representing the fragments are aligned using a sequence alignment program, such as "MACVECTOR" (IBI, New Haven, CT). *Corresponding* polypeptide fragments typically contain a similar, if not identical, number of residues. It will be understood, however, that corresponding fragments may contain insertions or deletions of residues with respect to one another, as well as some differences in their sequences.

30 A polypeptide sequence or fragment is *derived* from another polypeptide sequence or fragment when it has the same sequence of amino acid residues as the corresponding region of the fragment from which it is derived. A variant or variant sequence derived from a core sequence contains one of the sequences defined by the core sequence.

II. Ovine and Bovine Interferon- τ Genes

Ovine interferon- τ (OvIFN τ) is a major conceptus secretory protein produced by the embryonic trophectoderm during the critical period of maternal recognition in sheep. The isolation of OvIFN τ protein has been described (Godkin, *et al.*; Vallet, *et al.*, 1987, 1988).

5 One isolate of mature OvIFN τ is 172 amino acids in length (SEQ ID NO:2; exemplary nucleic acid coding sequence, SEQ ID NO:1). The cDNA coding sequence contains an additional 23 amino acids at the amino-terminal end of the mature protein (Imakawa, *et al.*, 1987). The coding sequence of this OvIFN τ isolate is presented as Figure 1.

10 Relative to other interferons, OvIFN τ shares the greatest sequence similarity with the interferon ω s (IFN ω s).

A homologous protein to OvIFN τ was isolated from cows (bIFN τ ; Helmer, *et al.*; Imakawa, *et al.*, 1989). OvIFN τ and BoIFN τ (i) have similar functions in maternal recognition of pregnancy, and (ii) share a high degree of amino acid and nucleotide sequence homology between mature proteins. The nucleic acid sequence homology between OvIFN τ and BoIFN τ is 76.3% for the 5' non-coding region, 89.7% for the coding region, and 91.9% for the 3' non-coding region. The amino acid sequence homology is 80.4%.

III. Isolation and Characterization of Human Interferon- τ Genes

20 A. Identification and Cloning of Human Genomic Sequences Encoding Interferon- τ Protein

Human DNA was screened for sequences homologous to interferon- τ (Example 1). Several sequences that hybridized with the OvIFN τ cDNA probe were identified. Several clones containing partial sequences of human interferon- τ were then isolated (Example 2). Two synthetic 25-mer oligonucleotides, corresponding to sequences from the OvIFN τ cDNA (Imakawa, *et al.*, 1987) were synthesized. These primers were employed in amplification reactions using DNA derived from the following two cDNA libraries: human term placenta and human term cytotrophoblast. The resulting amplified DNA fragments were electrophoretically separated and a band containing human IFN τ (HuIFN τ) amplification products was isolated. The amplification products were subcloned and the inserted amplification products
30 sequenced using the dideoxy termination method.

Comparison of sequences from five of these clones revealed a high degree of sequence homology between the isolates, but the sequences were not identical. This result suggests the existence of multiple variants of human interferon- τ genes. Analysis of the nucleotide and protein sequences suggests that human interferon- τ genes may be classified on the basis of
35 sequence homology into at least three groups. The groups are presented below.

Example 3 describes the isolation of several full-length human IFN γ genes. High molecular weight DNA was isolated from peripheral blood mononuclear cells (PBMCs) and size-fractionated. Fractions were tested for the presence of HuIFN γ sequences using polymerase chain reaction: DNA molecules from fractions that tested amplification positive
5 were used to generate a subgenomic library in λ gt11.

This subgenomic library was plated and hybridized with an OvIFN γ cDNA probe (Example 3A). Approximately 20 clones were identified that hybridized to the probe. Plaques corresponding to the positive clones were passaged, DNA isolated and analyzed by amplification reactions using OvIFN γ primers. Of these twenty plaques, six plaques generated
10 positive PCR signals. The phage from these six clones were purified and the inserts sequenced. One of the inserts from one of these six clones was used as a hybridization probe in the following screening.

Recombinant phage from the λ gt11 subgenomic library were screened using the human DNA hybridization probe just described (Example 3B). Five clones giving positive hybridization signals were isolated and the inserts sequenced. The sequences from three of the
15 clones overlapped, and the resulting consensus nucleic acid sequence (HuIFN γ 1) is presented as SEQ ID NO:3 with the predicted protein coding sequence presented as SEQ ID NO:4 (containing the leader sequence). The predicted mature protein coding sequence is presented as SEQ ID NO:30.

The sequences from the other two clones are presented as SEQ ID NO:15 (HuIFN γ 2) and SEQ ID NO:17 (HuIFN γ 3). The predicted mature amino acid sequence from HuIFN γ 2 is presented as SEQ ID NO:16. The predicted amino acid sequence from HuIFN γ 3 is presented as SEQ ID NO:18, and the mature amino acid sequence as SEQ ID NO:20.
20

For expression of recombinant HuIFN γ , a coding sequence can be placed in a number of bacterial expression vectors: for example, λ gt11 (Promega, Madison WI); pGEX (Smith, *et al.*, 1988); pGEMEX (Promega); and pBS (Stratagene, La Jolla CA) vectors. Other bacterial expression vectors containing suitable promoters, such as the T7 RNA polymerase promoter or the *tac* promoter, may also be used.
25

Yeast vectors can also be used in the practice of the present invention. They include
30 2 microns plasmid vectors (Ludwig, *et al.*), yeast integrating plasmids (YIps; *e.g.*, Shaw, *et al.*), YEP vectors (Shen, *et al.*), yeast centromere plasmids (YCps; *e.g.*, Ernst), and the like. Preferably, the vectors include an expression cassette containing an effective yeast promoter, such as the MF α 1 promoter (Ernst; Bayne, *et al.*), GADPH promoter (glyceraldehyde-3-phosphate-dehydrogenase; Wu, *et al.*), the galactose-inducible GAL10 promoter (Ludwig, *et*

al.; Feher, *et al.*; Shen, *et al.*), or the methanol-regulated alcohol oxidase (EWES) promoter. The EWES promoter is particularly useful in *Pocket pastoris* host cells (for example, the EWES promoter is used in pHIL and pPIC vectors included in the *Pocket* expression kit, available from Invitrogen, San Diego, CA).

5 The expression cassette may include additional elements to facilitate expression and purification of the recombinant protein, and/or to facilitate the insertion of the cassette into a vector or a yeast chromosome. For example, the cassette may include a signal sequence to direct secretion of the protein. An exemplary signal sequence suitable for use in a variety of yeast expression vectors is the MF α 1 pre-pro signal sequence (Bayne, *et al.*; Ludwig, *et al.*;
10 Shaw, *et al.*). Other signal sequences may also be used. For example, the Pho1 signal sequence (Elliot, *et al.*) is particularly effective in *Pocket Pastoris* and *Schizosaccharomyces pombe* host cells.

Exemplary expression cassettes include (i) a cassette containing (5' to 3') the EWES promoter, the Pho1 signal sequence, and a DNA sequence encoding HuIFN γ , for expression
15 in *P. pastoris* host cells, and (ii) a cassette containing (5' to 3') the MF α 1 promoter, the MF α 1 pre-pro signal sequence, and a DNA sequence encoding mature HuIFN γ , for expression in *S. cerevisiae* host cells.

Additional yeast vectors suitable for use with the present invention include, but are not limited to, other vectors with regulatable expression (Hitzeman, *et al.*; Rutter, *et al.*; Oeda, *et al.*).
20 *al.*). The yeast transformation host is typically *Saccharomyces cerevisiae*, however, as illustrated above, other yeast suitable for transformation can be used as well (*e.g.*, *Schizosaccharomyces pombe*, *Pocket pastoris* and the like).

The DNA encoding the HuIFN γ polypeptide can be cloned into any number of commercially available vectors to generate expression of the polypeptide in the appropriate host
25 system. These systems include the above described bacterial and yeast expression systems as well as the following: baculovirus expression (Reilly, *et al.*; Beames, *et al.*; Clontech, Palo Alto CA); plant cell expression, transgenic plant expression (*e.g.*, Gelvin and Schilperoort), and expression in mammalian cells (Clontech, Palo Alto CA; Gibco-BRL, Gaithersburg MD).

The recombinantly produced polypeptides can be expressed as fusion proteins or as
30 native proteins. The recombinantly produced polypeptides are typically isolated from lysed cells or culture media. The protein can be further purified after expression by standard methods, including size fractionation (column chromatography or preoperative gel electrophoresis) or affinity chromatography (using, for example, anti-HuIFN γ antibodies (solid

support available from Pharmacia, Piscataway NJ). Protein preparations can also be concentrated by, for example, filtration (Amicon, Danvers, Mass.).

Recombinant HuIFN τ obtained by such methods exhibits antiviral activity. Further, one advantage of HuIFN τ over other interferons (*e.g.*, IFN α) is that treatment of a subject with
5 therapeutic doses of HuIFN τ does not appear to be associated with cytotoxicity.

B. HuIFN τ Sequence Comparisons and Localization Studies

Comparison of the predicted protein sequences (Figure 2) of one of the human interferon- τ genes (SEQ ID NO:30) and the ovine interferon- τ gene demonstrates the levels of
10 sequence homology and divergence at the amino acid level.

An alignment of the nucleic acid sequences of the seven human interferon- τ nucleic acid sequences, described herein (Examples 2 and 3), with ovine interferon- τ is shown in Figures 3A and 3B. Sequences of OvIFN τ (oIFN τ), HuIFN τ 1, HuIFN τ 2, and HuIFN τ 3 start at the upper left corner of Figure 3A with the initiation ATG codon and continue through the second
15 page of the figure. Sequences of HuIFN τ 4, HuIFN τ 5, HuIFN τ 6 and HuIFN τ 7 start approximately half-way down Figure 3A with the CAG codon at amino acid position 40 (to the right of the exclamation marks) and continue through the second page of the figure. The 5' and 3' ends of each of the clones for HuIFN τ 4, HuIFN τ 5, HuIFN τ 6 and HuIFN τ 7 are represented by exclamation marks.

20 The complete coding sequence of OvIFN τ is presented in the top row of each aligned set. Nucleotides in the other sequences are indicated only at positions where they differ from those of OvIFN τ . Lower case letters represent nucleotide changes that do not result in amino acid changes, while upper case letters represent those changes that result in an amino acid substitution.

25 An alignment of the seven corresponding amino acid sequences, constructed in essentially the same manner as described above, is presented in Figures 4A and 4B. As above, the complete amino acid sequence of OvIFN τ is presented in the top row, and amino acids of other sequences are indicated only at positions where they differ from the ovine sequence.

An examination of the alignments reveals that the seven sequences may be grouped into
30 at least three groups. Group I contains HuIFN τ 1 and HuIFN τ 2, group II contains HuIFN τ 3, HuIFN τ 4 and HuIFN τ 5, and group III contains HuIFN τ 6 and HuIFN τ 7. These groups may represent families of interferon- τ genes having distinct cellular functions. The core polypeptide sequences defining the groups are presented herein as SEQ ID NO:31, SEQ ID NO:32 and

SEQ ID NO:33, respectively. A general core HuIFN γ amino acid sequence is presented herein as SEQ ID NO:34.

These groupings were established based on the following criteria. In mature proteins, Group I HuIFN γ s have an asparagine (ASN) at amino acid position number 95 (numbers in reference to Figures 4A to 4B), a methionine (MET) at amino acid position number 104, and a leucine (LEU) at amino acid position number 120; Group II HuIFN γ s have an aspartic acid (ASP) at amino acid position number 95, a threonine (THR) at amino acid position number 104, and a methionine (MET) at amino acid position number 120; and Group III HuIFN γ s have an arginine (ARG) at amino acid position number 72, a valine (VAL) at amino acid position number 120, and a serine (SER) at amino acid position number 122.

The nucleic acid and polypeptide human IFN γ sequences presented as SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20 can be used as the source for specific primers and probes to detect isolates of further human IFN γ coding sequences and/or pseudogenes. Further, as described above, there may be more than one isoform of the HuIFN γ protein and more than one coding sequence per species. The specific nucleic acid probes used in the practice of the present invention and antibodies reactive with the HuIFN γ polypeptides of the present invention may be useful to isolate unidentified variants of interferon- γ in mammals, according to the methods of the invention disclosed herein (*e.g.*, Examples 1 and 2).

The presence of HuIFN γ mRNA in human term placenta and amniocytes was analyzed. Results from these studies suggested that the presence of human IFN γ mRNA in the fetal-placental annex. The amniocytes also expressed the messages corresponding to OvIFN γ primers and a HuIFN γ probe, suggesting that the expression of HuIFN γ mRNA is not limited to the term placenta.

In addition, a RT-PCR analysis for the presence of HuIFN γ was applied to the total cellular RNA isolated from human adult lymphocytes: the results demonstrated that HuIFN γ mRNA exists in lymphocytes.

The expression of interferon- γ in human tissue was also examined using *in situ* hybridization; specific hybridization was observed in all term and first trimester placental tissues.

First trimester placental villi (composed of an outer layer of syncytiotrophoblast, an underlying layer of cytotrophoblast, and a central stromal region with various types of

mesenchymal cells) displayed the highest transcript level of HuIFN γ in the cytotrophoblast cells. Less intense but detectable levels were present in both the syncytiotrophoblast and stromal cells. A similar pattern of transcript expression was demonstrated in the placental villi of term tissue but the level of signal detection was low. First trimester extravillous trophoblasts displayed the highest amount of message and stained positive when present in the maternal blood spaces.

Howatson, *et al.*, noted IFN α production in the syncytiotrophoblast of chorionic villi in both first trimester and term tissues. Also, Paulesu, *et al.* observed IFN α in extravillous trophoblast as well as syncytiotrophoblasts, noting more intense and abundant reactivity in first trimester placental tissue when compared to those taken at term. These investigators employed antibodies raised against human IFN α subtypes, and none observed any IFN α in the villous cytotrophoblasts.

The human IFN γ gene appears to be highly expressed in early placental tissues (*e.g.*, first trimester placenta) by migrating extravillous trophoblasts, but is also expressed in villous syncytiotrophoblasts, villous cytotrophoblasts, and various stromal cells.

IV. Human Interferon- γ Polypeptide Fragments and Protein Modifications

A. HuIFN γ Polypeptide Fragments

Portions of the HuIFN γ interferon molecule may be used to substitute regions of other interferon molecules. For example, the region of an interferon alpha molecule that is responsible for increased cytotoxicity, relative to HuIFN γ treatment, can be identified by substituting polypeptide regions derived from HuIFN γ for regions of an interferon alpha molecule. Such substitutions can be carried out by manipulation of synthetic genes (see below) encoding the selected HuIFN γ and interferon alpha molecules, coupled to the functional assays, such as, antiviral, antiproliferative and cytotoxicity assays (*e.g.*, WO 94/10313, published 11 May 1994). Exemplary HuIFN γ polypeptide fragments include, but are not limited to, the polypeptides presented as SEQ ID NO:21 to SEQ ID NO:26.

Synthetic gene constructs facilitate introduction of mutations for possible enhancement of antitumor (anticellular proliferative) and antiviral activities. Further, the disparate regions of the molecule responsible for different functions allows for separate manipulation of different functions.

Synthetic peptides can be generated which correspond to the HuIFN γ polypeptides of the present invention. Synthetic peptides can be commercially synthesized or prepared using standard methods and apparatus in the art (Applied Biosystems, Foster City CA).

Alternatively, oligonucleotide sequences encoding peptides can be either synthesized directly by standard methods of oligonucleotide synthesis, or, in the case of large coding sequences, synthesized by a series of cloning steps involving a tandem array of multiple oligonucleotide fragments corresponding to the coding sequence (Crea; Yoshio *et al.*; Eaton
5 *et al.*). Oligonucleotide coding sequences can be expressed by standard recombinant procedures (Maniatis *et al.*; Ausubel, *et al.*).

The biological activities of the interferon- τ polypeptides described above can be exploited using either the human interferon- τ polypeptides alone or conjugated with other proteins (see below).

10

B. Production of Fusion Proteins

In another aspect, the present invention includes human interferon- τ or human interferon- τ -derived polypeptides covalently attached to a second polypeptide to form a fused, or hybrid, protein. The human interferon- τ sequences making up such fused proteins can be
15 recombinantly-produced-interferon- τ or a bioactive portion thereof, as described above.

For example, where human interferon- τ is used to inhibit viral expression, polypeptides derived from HuIFN τ demonstrating antiviral activity may be advantageously fused with a soluble peptide, such as, serum albumin, an antibody (*e.g.*, specific against an virus-specific cell surface antigen), or an interferon alpha polypeptide.

20 In one embodiment, the HuIFN τ polypeptides provide a method of reducing the toxicity of other interferon molecules (*e.g.*, IFN β or IFN α) by replacing toxicity-associated regions of such interferons with, for example, corresponding interferon- τ regions having lower toxicity.

In another embodiment, fusion proteins are generated containing human interferon- τ regions that have anticellular proliferation properties. Such regions may be obtained from, for
25 example, the human interferon- τ sequences disclosed herein.

The fused proteins of the present invention may be formed by chemical conjugation or by recombinant techniques. In the former method, the human interferon- τ and second selected polypeptide are modified by conventional coupling agents for covalent attachment. In one exemplary method for coupling soluble serum albumin to a human interferon- τ polypeptide,
30 serum albumin is derivatized with N-succinimidyl-S-acetyl thioacetate (Duncan), yielding thiolated serum albumin. The activated serum albumin polypeptide is then reacted with human interferon- τ derivatized with N-succinimidyl 3-(2-pyridyldithio) propionate (Cumber), to produce the fused protein joined through a disulfide linkage.

As an alternative method, recombinant human interferon- τ may be prepared with a cysteine residue to allow disulfide coupling of the human interferon- τ to an activated ligand, thus simplifying the coupling reaction. An interferon- τ expression vector, used for production of recombinant human interferon- τ , can be modified for insertion of an internal or a terminal
5 cysteine codon according to standard methods of site-directed mutagenesis (Ausubel, *et al.*).

In one method, a fused protein is prepared recombinantly using an expression vector in which the coding sequence of a second selected polypeptide is joined to the human interferon- τ coding sequence. For example, human serum albumin coding sequences can be fused in-frame to the coding sequence of a human interferon- τ polypeptide, such as, SEQ ID
10 NO:25. The fused protein is then expressed using a suitable host cell. The fusion protein may be purified by molecular-sieve and ion-exchange chromatography methods, with additional purification by polyacrylamide gel electrophoretic separation and/or HPLC chromatography, if necessary.

It will be appreciated from the above how human interferon- τ -containing fusion proteins
15 may be prepared. One variation on the above fusion is to exchange positions of the human interferon- τ and selected second protein molecules in the fusion protein (*e.g.*, carboxy terminal versus amino terminal fusions). Further, internal portions of a native human interferon- τ polypeptide (for example, amino acid regions of between 20 and 172 amino acids) can be assembled into polypeptides where two or more such human interferon- τ portions are
20 contiguous that are normally discontinuous in the native protein.

In addition to the above-described fusion proteins, the present invention also contemplates polypeptide compositions having (a) a human interferon- τ polypeptide, where said polypeptide is (i) derived from the N-terminal portion of a human interferon- τ amino acid coding sequence, and (ii) between 8 and 37 amino acids long, and (b) a second soluble
25 polypeptide. Interferon- α and interferon- β are examples of such second soluble polypeptides. Such hybrid interferon compositions can be encoded by hybrid nucleic acid molecules formed of a 5' end segment that encodes the N-terminal amino acid sequence of an interferon-tau polypeptide, and a 3' end segment that encodes the C-terminal amino acid sequence of a non-tau interferon type I polypeptide. The two segments are spliced in a region corresponding to
30 the portion of a mature interferon polypeptide between about amino acid residues 8 and 37.

The hybrid HuIFN τ polypeptides associated with reduced toxicity may be co-administered with or substituted for more toxic interferons to reduce the toxicity of the more toxic interferons when used in pharmaceutical formulations or in therapeutic applications.

C. Antibodies Reactive with Human Interferon- γ

Fusion proteins containing the polypeptide antigens of the present invention fused with the glutathione-S-transferase (Sj26) protein can be expressed using the pGEX-GLI vector system in *E. coli* JM101 cells. The fused Sj26 protein can be isolated readily by glutathione substrate affinity chromatography (Smith, *et al.*, 1988). Expression and partial purification of HuIFN γ proteins is described in (Example 5), and is applicable to any of the other soluble, induced polypeptides coded by sequences described by the present invention.

Insoluble GST (sj26) fusion proteins can be purified by preparative gel electrophoresis.

Alternatively, HuIFN γ - β -galactosidase fusion proteins can be isolated as described in Example 4.

Also included in the invention is an expression vector, such as the lambda gt11 or pGEX vectors described above, containing HuIFN γ coding sequences and expression control elements which allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation codon, and translation and transcription termination sequences, and an insertion site for introducing the insert into the vector.

The DNA encoding the desired polypeptide can be cloned into any number of vectors (discussed above) to generate expression of the polypeptide in the appropriate host system. These recombinant polypeptides can be expressed as fusion proteins or as native proteins.

In another aspect, the invention includes specific antibodies directed against the polypeptides of the present invention. Typically, to prepare antibodies, a host animal, such as a rabbit, is immunized with the purified antigen or fused protein antigen. Hybrid, or fused, proteins may be generated using a variety of coding sequences derived from other proteins, such as β -galactosidase or glutathione-S-transferase. The host serum or plasma is collected following an appropriate time interval, and this serum is tested for antibodies specific against the antigen. Example 5 describes the production of rabbit serum antibodies which are specific against the HuIFN γ antigens in a Sj26/HuIFN γ hybrid protein. These techniques can be applied to the all of the HuIFN γ molecules and polypeptides derived therefrom.

The gamma globulin fraction or the IgG antibodies of immunized animals can be obtained, for example, by use of saturated ammonium sulfate or DEAE Sephadex, or other techniques known to those skilled in the art for producing polyclonal antibodies.

Alternatively, purified protein or fused protein may be used for producing monoclonal antibodies. Here the spleen or lymphocytes from a animal immunized with the selected polypeptide antigen are removed and immortalized or used to prepare hybridomas by methods known to those skilled in the art (Harlow, *et al.*). Lymphocytes can be isolated from a

peripheral blood sample. Epstein-Barr virus (EBV) can be used to immortalize human lymphocytes or a fusion partner can be used to produce hybridomas.

Antibodies secreted by the immortalized cells are screened to determine the clones that secrete antibodies of the desired specificity, for example, by using the ELISA or Western blot
5 method (Ausubel, *et al.*).

Antigenic regions of polypeptides are generally relatively small, typically 7 to 10 amino acids in length. Smaller fragments have been identified as antigenic regions. Human interferon- τ polypeptide antigens are identified as described above. The resulting DNA coding regions can be expressed recombinantly either as fusion proteins or isolated polypeptides.

10 In addition, some amino acid sequences can be conveniently chemically synthesized (Applied Biosystems, Foster City CA). Antigens obtained by any of these methods may be directly used for the generation of antibodies or they may be coupled to appropriate carrier molecules. Many such carriers are known in the art and are commercially available (*e.g.*, Pierce, Rockford IL).

15 Antibodies reactive with HuIFN τ are useful, for example, in the analysis of structure/function relationships.

V. Utility

A. Reproductive

20 Although HuIFN τ bears some similarity to the IFN α family based on structure and its potent antiviral properties, the IFN α s do not possess the reproductive properties associated with HuIFN τ . Also, recombinant bovine IFN α has little or no effect on interestrus interval compared to IFN τ (Davis, *et al.*, 1992).

Therefore, although HuIFN τ has some structural similarities to other interferons, it has
25 very distinctive properties of its own: for example, the capability of significantly influencing the biochemical events of the estrous cycle.

The human IFN τ of the present invention can be used in methods of enhancing fertility and prolonging the life span of the *corpus luteum* in female mammals as generally described in Hansen, *et al.*, herein incorporated by reference. Further, the human interferon- τ of the
30 present invention could be used to regulate growth and development of uterine and/or fetal-placental tissues. HuIFN τ is particularly useful for treatment of humans, since potential antigenic responses are less likely using such a same-species protein.

B. Antiviral Properties

The antiviral activity of HuIFN γ has broad therapeutic applications without the toxic effects that are usually associated with IFN α s. HuIFN γ was found to exert its antiviral activity without adverse effects on the cells: no evidence of cytotoxic effects attributable to the administration of HuIFN γ was observed. It is the lack of cytotoxicity of HuIFN γ which makes it extremely valuable as an *in vivo* therapeutic agent. This lack of cytotoxicity sets HuIFN γ apart from most other known antiviral agents and all other known interferons.

HuIFN γ is an effective antiviral agent against a wide variety of viruses, including both RNA and DNA viruses. Human interferon- γ may be used for the treatment of, for example, the following viral diseases: human immunodeficiency virus (HIV), hepatitis c virus (HCV) and hepatitis B virus (HBV). Formulations comprising the HuIFN γ compounds of the present invention can be used to inhibit viral replication.

The human IFN γ of the present invention can be employed in methods for affecting the immune relationship between fetus and mother, for example, in preventing transmission of maternal viruses (*e.g.*, HIV) to the developing fetus. The human interferon- γ is particularly useful for treatment of humans, since potential antigenic responses are less likely using a homologous protein.

C. Anticellular Proliferation Properties

IFN γ exhibits antiproliferative activity against human tumor cells without toxicity and is as potent or more potent than human IFN α . Clinical trials of the IFN α 's have shown them to be effective antitumor agents (Dianzani; Krown). One therapeutic advantage of HuIFN γ as a therapeutic is the elimination of toxic effects seen with high doses IFN α s.

One application of the HuIFN γ is against tumors like Kaposi's sarcoma (associated with HIV infection) where the antineoplastic effects of HuIFN γ are coupled with HuIFN γ ability to inhibit retroviral growth.

HuIFN γ exhibits potent anticellular proliferation activity. HuIFN γ can also be used to inhibit cellular growth without the negative side effects associated with other interferons which are currently known. Formulations comprising the HuIFN γ compounds of the subject invention can be used in methods to inhibit, prevent, slow or reduced tumor cell growth, including, but are not limited to, the following types of tumor cells: human carcinoma cells, hematopoietic cancer cells, human leukemia cells, human lymphoma cells, human melanoma cells and steroid-sensitive tumor cells (for example, mammary tumor cells/estrogen-dependent tumor cells).

D. Interfering with the Binding of Interferons to Receptors

IFN γ appears to interact with the Type I IFN receptor via several epitopes on the molecule, and these regions either separately or in combination may differently affect distinct functions of HuIFN γ . The polypeptides of the present invention may be useful for the selective inhibition of binding of interferons to the interferon receptor.

Accordingly, HuIFN γ polypeptides may be used as immunoregulatory molecules when it is desired to prevent immune responses triggered by interferon molecules. Such peptides could be used as immunosuppressants to prevent, for example, interferon-mediated immune responses to tissue transplants. Other types of interferon mediated responses may also be blocked, such as the cytotoxic effects of alpha interferon.

E. Pharmaceutical Compositions

One advantage, for therapeutic applications, of HuIFN γ over other interferons, such as IFN α and IFN β , is that treatment with therapeutic doses of HuIFN γ does not appear to be associated with cytotoxicity. In particular, HuIFN γ appears to be non-toxic at concentrations at which IFN β induces toxicity.

HuIFN γ proteins can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations comprising interferons or interferon-like compounds have been previously described (for example, Martin). In general, the compositions of the subject invention will be formulated such that an effective amount of the HuIFN γ is combined with a suitable carrier in order to facilitate effective administration of the composition.

The compositions used in these therapies may also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, suppositories, injectable, and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and adjuvants which are known to those of skill in the art. Preferably, the compositions of the invention are in the form of a unit dose and will usually be administered to the patient one or more times a day.

HuIFN γ , or related polypeptides, may be administered to a patient (or subject in need of treatment) in any pharmaceutically acceptable dosage form, including intravenous, intramuscular, intralesional, or subcutaneous injection. Specifically, compositions and methods used for other interferon compounds can be used for the delivery of these compounds.

One primary advantage of the compounds of the subject invention, however, is the extremely low cytotoxicity of the HuIFN γ proteins. Because of this low cytotoxicity, it is possible to administer the HuIFN γ in concentrations which are greater than those which can generally be utilized for other interferon (*e.g.*, IFN α) compounds. Thus, HuIFN γ can be
5 administered at rates from about 5×10^4 to 20×10^6 units/day to about 500×10^6 units/day or more. In a preferred embodiment, the dosage is about 20×10^6 units/day. High doses are preferred for systemic administration. It should, of course, be understood that the compositions and methods of this invention may be used in combination with other therapies.

Once improvement of a patient's condition has occurred, a maintenance dose is
10 administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

15 The compositions of the subject invention can be administered through standard procedures to treat a variety of cancers and viral diseases including those for which other interferons have previously shown activity. See, for example, Finter, *et al.*; Dianzani; Francis, *et al.* and U.S. Patent Nos. 4,885,166 and 4,975,276. However, as discussed above, the compositions of the subject invention have unique features and advantages, including their
20 ability to treat these conditions without toxicity.

F. Treatment of Skin Disorders

Disorders of the skin can be treated intralesionally using HuIFN γ , wherein formulation and dose will depend on the method of administration and on the size and severity of the lesion
25 to be treated. Preferred methods include intradermal and subcutaneous injection. Multiple injections into large lesions may be possible, and several lesions on the skin of a single patient may be treated at one time. The schedule for administration can be determined by a person skilled in the art. Formulations designed for sustained release can reduce the frequency of administration.

30

G. Systemic Treatment

Systemic treatment is essentially equivalent for all applications. Multiple intravenous, subcutaneous and/or intramuscular doses are possible, and in the case of implantable methods

for treatment, formulations designed for sustained release are particularly useful. Patients may also be treated using implantable subcutaneous portals, reservoirs, or pumps.

H. Regional Treatment

5 Regional treatment with the HuIFN γ polypeptides of the present invention is useful for treatment of cancers in specific organs. Treatment can be accomplished by intraarterial infusion. A catheter can be surgically or angiographically implanted to direct treatment to the affected organ. A subcutaneous portal, connected to the catheter, can be used for chronic treatment, or an implantable, refillable pump may also be employed.

10

The following examples illustrate, but in no way are intended to limit the present invention.

Materials and Methods

15 Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, *Taq* DNA polymerase, and calf intestinal phosphatase were purchased from New England Biolabs (Beverly, MA) or Promega Biotech (Madison, WI): these reagents were used according to the manufacturer's instruction. For sequencing reactions, a "SEQUENASE DNA II" sequencing kit was used (United States Biochemical Corporation, Cleveland OH). Immunoblotting and
20 other reagents were from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Needham, MA). Nitrocellulose filters are obtained from Schleicher and Schuell (Keene, NH).

Synthetic oligonucleotide linkers and primers are prepared using commercially available automated oligonucleotide synthesizers (*e.g.*, an ABI model 380B-02 DNA synthesizer (Applied Biosystems, Foster City, CA)). Alternatively, custom designed synthetic oligonucleotides may
25 be purchased, for example, from Synthetic Genetics (San Diego, CA). cDNA synthesis kit and random priming labeling kits are obtained from Boehringer-Mannheim Biochemical (BMB, Indianapolis, IN).

Oligonucleotide sequences encoding polypeptides can be either synthesized directly by standard methods of oligonucleotide synthesis, or, in the case of large coding sequences,
30 synthesized by a series of cloning steps involving a tandem array of multiple oligonucleotide fragments corresponding to the coding sequence (Crea; Yoshio, *et al.*; Eaton *et al.*). Oligonucleotide coding sequences can be expressed by standard recombinant procedures (Maniatis, *et al.*; Ausubel, *et al.*; Rothstein).

Alternatively, peptides can be synthesized directly by standard *in vitro* techniques (Applied Biosystems, Foster City CA).

Common manipulations involved in polyclonal and monoclonal antibody work, including antibody purification from sera, are performed by standard procedures (Harlow, *et al.*). Pierce (Rockford, IL) is a source of many antibody reagents.

Recombinant human IFN α (rHuIFN α) and rBoIFN γ was obtained from Genentech Inc. (South San Francisco, CA). The reference preparation of recombinant human IFN α (rHuIFN α) was obtained from the National Institutes of Health: rHuIFN α is commercially available from Lee Biomolecular (San Diego, CA).

10 All tissue culture media, sera and IFNs used in this study were negative for endotoxin, as determined by assay with Limulus amoebocyte lysate (Associates of Cape Cod, Woods Hole, MA) at a sensitivity level of 0.07 ng/ml.

A. General ELISA Protocol for Detection of Antibodies

15 Polystyrene 96 well plates Immulon II (PGC) were coated with 5 μ g/mL (100 μ L per well) antigen in 0.1 M carb/bicarbonate buffer, pH 9.5. Plates were sealed with parafilm and stored at 4°C overnight.

Plates were aspirated and blocked with 300 μ L 10% NGS and incubated at 37°C for 1 hr.

20 Plates were washed 5 times with PBS 0.5% "TWEEN-20".

Antisera were diluted in 0.1 M PBS, pH 7.2. The desired dilution(s) of antisera (0.1 mL) were added to each well and the plate incubated 1 hours at 37°C. The plates was then washed 5 times with PBS 0.5% "TWEEN-20".

25 Horseradish peroxidase (HRP) conjugated goat anti-human antiserum (Cappel, Durham, NC) was diluted 1/5,000 in PBS. 0.1 mL of this solution was added to each well. The plate was incubated 30 min at 37°C, then washed 5 times with PBS.

Sigma ABTS (substrate) was prepared just prior to addition to the plate.

30 The reagent consists of 50 mL 0.05 M citric acid, pH 4.2, 0.078 mL 30% hydrogen peroxide solution and 15 mg ABTS. 0.1 mL of the substrate was added to each well, then incubated for 30 min at room temperature. The reaction was stopped with the addition of 0.050 mL 5% SDS (w/v). The relative absorbance is determined at 410 nm.

EXAMPLE 1

Southern Blot Analysis of Human
High Molecular Weight DNA

Human venous blood samples from healthy donors were collected in heparinized tubes
5 and peripheral blood lymphocytes were isolated by density-gradient centrifugation using a
Ficoll-Isopaque gradient (1.077 g/ml) (Sigma Chemical Co.). High molecular weight (HMW)
DNA was isolated from these cells (Sambrook, *et al.*).

Two 10 μ g samples of HMW DNA were digested with the restriction endonucleases
*Hind*III or *Pst*I (Promega) for 2 hours at 37°C, and the DNA fragments electrophoretically
10 separated in a 0.8% agarose gel (Bio-Rad, Richmond, CA) at 75 volts for 8 hours. The DNA
fragments were transferred onto a nylon membrane (IBI-International Biotechnologies, Inc.,
New Haven, CT). The membrane was baked at 80°C for 2 hours and incubated at 42°C for
4 hours in the following pre-hybridization solution: 5 \times SSC (1 \times SSC is 0.15 M NaCl and
0.15 M sodium citrate), 50% vol/vol formamide, 0.6% (wt/vol) SDS, 0.5% (wt/vol) nonfat
15 dry milk, 20 mM Tris-HCl (pH 7.5), 4 mM EDTA, and 0.5 mg/ml single stranded herring
sperm DNA (Promega).

The filter was then incubated in a hybridization solution (5 \times SSC, 20% vol/vol
formamide, 0.6% (wt/vol) SDS, 0.5% (wt/vol) nonfat dry milk, 20 mM Tris-HCl (pH 7.5),
4 mM EDTA, and 2 \times 10⁸ cpm/ml ³²P-labelled OvIFN γ cDNA (Imakawa, *et al.*, 1987)) for
20 18 hours at 42°C. The filter was washed at 42°C for 15 minutes with 2 \times SSC and 0.1%
(wt/vol) SDS and exposed to X-ray film (XAR, Eastman Kodak, Rochester, NY) at -80°C for
48 hours in the presence of an intensifying screen.

Autoradiography detected a hybridization signal at approximately 3.4 kb in DNA
digested with *Pst*I and a slightly smaller (\approx 3.0 kb) fragment in the *Hind*III digested DNA.
25 These results indicate the presence of human DNA sequences complementary to the OvIFN γ
cDNA probe.

EXAMPLE 2

Isolation of Partial Sequence of
Human IFN γ cDNA by PCR

30

Two synthetic oligonucleotides (each 25-mer), corresponding to the sequence 231 to
255 (contained in SEQ ID NO:5) and 566 to 590 (contained in SEQ ID NO:6) of OvIFN γ
cDNA (numbering relative to the cap site, Imakawa, *et al.*, 1987) were synthesized. These
primers contained, respectively, cleavage sites for the restriction endonucleases *Pst*I and *Eco*RI.
35 SEQ ID NO:5 was modified to contain the *Eco*RI site, which begins at position 569.

DNA was isolated from approximately 1×10^5 plaque forming units (pfu) of the following two cDNA libraries: human term placenta (Clontech, Inc., Palo Alto, CA) and human term cytotrophoblast (Dr. J.F. Strauss, University of Pennsylvania, Philadelphia PA).

The DNA was employed in polymerase chain reaction (PCR) amplifications (Mullis; Mullis, *et al.*; Perkin Elmer Cetus Corp. Norwalk CT). Amplification reactions were carried out for 30 cycles (45°C, 1m; 72°C, 2m; 94°C, 1m) (thermal cycler and reagents, Perkin Elmer Cetus) using primers SEQ ID NO:5/SEQ ID NO:6.

Amplification products were electrophoretically separated (100 volts in a 1.5 % agarose gel (Bio-Rad)) and transferred onto a nylon membrane (IBI). The membrane was baked at 80°C for 2 hours and pre-hybridized and hybridized with ³²P-labelled OvIFN γ cDNA as described above. The membrane was washed in $5 \times$ SSC/0.1 % (wt/vol) SDS for 5 minutes at 42°C and in $2 \times$ SSC/0.1 % (wt/vol) SDS for 2 minutes at 42°C. It was then exposed at -80°C to "XAR" (Eastman Kodak) X-ray film for 24 hours in the presence of an intensifying screen. An amplification product that hybridized with the labelled probe DNA was detected.

PCR was performed again as directed above. Amplified products were digested with the restriction endonucleases *EcoRI* and *PstI* (Promega) for 90 minutes at 37°C. The resulting DNA fragments were electrophoretically separated as described above and the band containing the HuIFN γ amplification product was excised from the gel. DNA fragments were recovered by electroelution, subcloned into *EcoRI/PstI* digested-dephosphorylated plasmid pUC19 and transformed into *E. coli* strain JM101 (Promega) by calcium chloride method (Sambrook, *et al.*). The plasmids were isolated and the inserted amplification product sequenced using the dideoxy termination method (Sanger, *et al.*; "SEQUENASE" reactions, United States Biochemical, Cleveland, OH). Nucleotide sequences were determined, and comparison of these as well as the deduced amino acid sequences to other IFN sequences were performed using "DNA STAR SOFTWARE" (Madison, WI).

Comparison of the sequences of these clones revealed the following five different clones: from the human placental library, HuIFN γ 6 (299 bp; SEQ ID NO:7, SEQ ID NO:8), HuIFN γ 7 (288 bp; SEQ ID NO:9, SEQ ID NO:10) and HuIFN γ 4 (307 bp; SEQ ID NO:11, SEQ ID NO:12), which exhibit 95% identity in their nucleotide sequences; from the cytotrophoblast library clone CTB 35 (HuIFN γ 5; 294 basepairs; SEQ ID NO:13, SEQ ID NO:14), which shares 95% and 98% identity with HuIFN γ 6 and HuIFN γ 4, respectively.

EXAMPLE 3

Isolation of Full-Length Human IFN γ Genes

Ten micrograms PBMC HMW DNA was digested with restriction endonuclease *EcoRI* and subjected to electrophoretic analysis in a 0.8% agarose gel. A series of samples containing
5 ranges of DNA fragments sized 1.5 to 10 kb (e.g., 1.5 to 2.5 kb, 2.5 kb to 3 kb) were excised from the gel. The DNAs were electroeluted and purified. Each DNA sample was amplified as described above using the OvIFN γ primers. The DNA molecules of any sample that yielded a positive PCR signal were cloned into λ gt11 (the subgenomic λ gt11 library).

10 A. PCR Identification of Clones Containing Sequences Complementary to OvIFN γ

The λ gt11 phage were then plated for plaques and plaque-lift hybridization performed using the 32 P-labelled OvIFN γ cDNA probe. Approximately 20 clones were identified that hybridized to the probe.

Plaques that hybridized to the probe were further analyzed by PCR using the OvIFN γ
15 primers described above. Six plaques which generated positive PCR signals were purified. The phage DNA from these clones was isolated and digested with *EcoRI* restriction endonuclease. The DNA inserts were subcloned into pUC19 vectors and their nucleotide sequences determined by dideoxy nucleotide sequencings.

20 B. Hybridization Identification of Clones Containing Sequences Complementary to PCR-Positive Phage

Recombinant phage from the λ gt11 subgenomic library were propagated in *E. coli* Y1080 and plated with *E. coli* Y1090 at a density of about 20,000 plaques/150 mm plate. The plates were overlaid with duplicate nitrocellulose filters, which were hybridized with a 32 P-
25 labelled probe from one of the six human IFN γ cDNA clones isolated above.

Clones giving positive hybridization signals were further screened and purified. The phage DNAs from hybridization-positive clones were isolated, digested with *EcoRI*, subcloned into pUC19 vector and sequenced. The sequence information was then analyzed.

30 1. HuIFN γ 1. Three clones yielded over-lapping sequence information for over 800 bases relative to the mRNA cap site (clones were sequenced in both orientations). The combined nucleic acid sequence information is presented as SEQ ID NO:3 and the predicted protein coding sequence is presented as SEQ ID NO:4. Comparison of the predicted mature protein sequence (SEQ ID NO:30) of this gene to the predicted protein sequence of
35 OvIFN γ is shown in Figure 2.

2. HuIFN γ 2, HuIFN γ 3. Two additional clones giving positive hybridization signals (HuIFN γ 2 and HuIFN γ 3) were also screened, purified, and phage DNAs subcloned and sequenced as above. The sequences of these two clones are presented in Figures 3A and 3B. As can be appreciated in Figures 3A and 3B, the nucleotide sequence of both clones (HuIFN γ 2 and HuIFN γ 3) is homologous to that of HuIFN γ 1 and OvIFN γ .

HuIFN γ 2 (SEQ ID NO:15), may be a pseudo-gene, as it appears to contain a stop codon at position 115-117. The sequence, SEQ ID NO:15, is presented without the leader sequence. The leader sequence is shown in Figure 4A. As can be seen from the HuIFN γ 2 sequence presented in Figure 4A, the first amino acid present in mature HuIFN γ 1 (a CYS residue) is not present in the HuIFN γ 2 sequence. Accordingly, the predicted amino acid sequence presented as SEQ ID NO:16 corresponds to a mature HuIFN γ protein with the exceptions of the first CYS residue and the internal stop codon.

The internal stop codon in the nucleic acid coding sequence can be modified by standard methods to replace the stop codon with an amino acid codon, for example, encoding GLN. The amino acid GLN is present at this position in the other isolates of human IFN γ (HuIFN γ). Standard recombinant manipulations also allow introduction of the initial CYS residue if so desired.

HuIFN γ 3 (SEQ ID NO:17), appears to encode a human IFN γ protein. The translated amino acid sequence of the entire protein, including the leader sequence, is presented as SEQ ID NO:18. The translated amino acid sequence of the mature protein is presented as SEQ ID NO:20 (nucleic acid sequence, SEQ ID NO:19).

EXAMPLE 4

Isolation of Human Interferon- γ Fusion Protein

Sepharose 4B beads conjugated with anti-beta galactosidase is purchased from Promega. The beads are packed in 2 ml column and washed successively with phosphate-buffered saline with 0.02% sodium azide and 10 ml TX buffer (10 mM Tris buffer, pH 7.4, 1% aprotinin).

The HuIFN γ coding sequence (*e.g.*, SEQ ID NO:19, *i.e.*, minus the nucleotides corresponding to the leader sequence) is cloned into the polylinker site of lambda gt11. The HuIFN γ coding sequence is placed in-frame with the amino terminal β -galactosidase coding sequences in lambda gt11. Lysogens infected with gt11/HuIFN γ are used to inoculate 500 ml of NZYDT broth. The culture is incubated at 32°C with aeration to an O.D. of about 0.2 to 0.4, then brought to 43°C quickly in a 43°C water bath for 15 minutes to induce gt11 peptide synthesis, and incubated further at 37°C for 1 hour. The cells are pelleted by centrifugation,

suspended in 10 ml of lysis buffer (10 mM Tris, pH 7.4 containing 2% "TRITON X-100" and 1% aprotinin added just before use.

The resuspended cells are frozen in liquid nitrogen then thawed, resulting in substantially complete cell lysis. The lysate is treated with DNaseI to digest bacterial and phage DNA, as evidenced by a gradual loss of viscosity in the lysate. Non-solubilized material is removed by centrifugation.

The clarified lysate material is loaded on the Sepharose column, the ends of the column closed, and the column placed on a rotary shaker for 2 hrs. at room temperature and 16 hours at 4°C. After the column settles, it is washed with 10 ml of TX buffer. The fused protein is eluted with 0.1 M carbonate/bicarbonate buffer, pH10. Typically, 14 ml of the elution buffer is passed through the column, and the fusion protein is eluted in the first 4-6 ml of eluate.

The eluate containing the fusion protein is concentrated in "CENTRICON-30" cartridges (Amicon, Danvers, Mass.). The final protein concentrate is resuspended in, for example, 400 µl PBS buffer. Protein purity is analyzed by SDS-PAGE.

For polyclonal antibodies, the purified fused protein is injected subcutaneously in Freund's adjuvant in a rabbit. Approximately 1 mg of fused protein is injected at days 0 and 21, and rabbit serum is typically collected at 6 and 8 weeks.

EXAMPLE 5

Preparation of Anti-HuIFN γ Antibody

A. Expression of Glutathione-S-Transferase Fusion Proteins.

The HuIFN γ coding sequence (*e.g.*, SEQ ID NO:19) is cloned into the pGEX vector (Boyer, *et al.*; Frangioni, *et al.*; Guan, *et al.*; Hakes, *et al.*; Smith, *et al.*, 1988). The pGEX vector (Smith, *et al.*, 1988) was modified by insertion of a thrombin cleavage sequence in-frame with the glutathione-S-transferase protein (GST -- sj26 coding sequence). This vector is designated pGEXthr. The HuIFN γ coding sequence is placed in-frame with the sj26-thrombin coding sequences (Guan, *et al.*; Hakes, *et al.*). The HuIFN γ coding sequence insert can be generated by the polymerase chain reaction using PCR primers specific for the insert.

The HuIFN γ fragment is ligated to the linearized pGEXthr vector. The ligation mixture is transformed into *E. coli* and ampicillin resistant colonies are selected. Plasmids are isolated from the ampicillin resistant colonies and analyzed by restriction enzyme digestion to identify clones containing the HuIFN γ insert (vector designated pGEXthr-HuIFN γ).

E. coli strain XL-I Blue is transformed with pGEXthr-HuIFN γ and is grown at 37°C overnight. DNA is prepared from randomly-picked colonies. The presence of the insert

coding sequence is typically confirmed by (i) restriction digest mapping, (ii) hybridization screening using labelled HuIFN γ probes (*i.e.*, Southern analysis), or (iii) direct DNA sequence analysis.

5 B. Partial Purification of Fusion Proteins

A pGEXthr-HuIFN γ clone is grown overnight. The overnight culture is diluted 1:10 with LB medium containing ampicillin and grown for one hour at 37°C. Alternatively, the overnight culture is diluted 1:100 and grown to OD of 0.5-1.0 before addition of IPTG (isopropylthio- β -galactoside). IPTG (GIBCO-BRL, Gaithersburg MD) is added to a final
10 concentration of 0.2-0.5 mM for the induction of protein expression and the incubation is typically continued for 2-5 hours, preferably 3.5 hours.

Bacterial cells are harvested by centrifugation and resuspended in 1/100 culture volume of MTPBS (150 mM NaCl, 16 mM Na₂HP0₄, 4 mM NaH₂PO₄). Cells are lysed by lysozyme, sonication or French press, and lysates cleared of cellular debris by centrifugation.

15 An aliquot of the supernatant obtained from IPTG-induced cultures of pGEXthr-HuIFN γ -containing cells and an aliquot of the supernatant obtained from IPTG-induced cultures of pGEXthr-vector alone are analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blotting, as described below.

If necessary, the extracts can be concentrated by ultrafiltration using, for example, a
20 "CENTRICON 10" filter.

Alternatively, the fusion proteins are partially purified over a glutathione agarose affinity column as described in detail by Smith, *et al.*, 1988. In this method, 100 ml cultures are grown overnight. The cultures are diluted to 1 liter, and the cells grown another hour at 37°C. Expression of the fusion proteins is induced using IPTG. The induced cultures are
25 grown at 37°C for 3.5 hours. Cells are harvested and a sonicator used to lyse the cells. Cellular debris is pelleted and the clear lysate loaded onto a glutathione "SEPHAROSE" column. The column is washed with several column volumes. The fusion protein is eluted from the affinity column with reduced glutathione and dialyzed. The HuIFN γ can be liberated from the hybrid protein by treatment with thrombin. The sj26 and HuIFN γ fragments of the
30 hybrid protein can then be separated by size fractionation over columns or on gels.

Alternatively, the HuIFN γ portion of the hybrid protein is released from the column by treatment with thrombin (Guan, *et al.*; Hakes, *et al.*).

C. Antibodies Against the Fusion Protein

The purified Sj26/HuIFN γ fused protein is injected subcutaneously in Freund's adjuvant in a rabbit. Approximately 1 mg of fused protein is injected at days 0 and 21, and rabbit serum is typically collected at 6 and 8 weeks. A second rabbit is similarly immunized with
5 purified Sj26 protein obtained from control bacterial lysate.

Minilysates from the following bacterial cultures are prepared: (1) KM392 cells infected with pGEXthr and pGEXthr containing the HuIFN γ insert; and (2) cells infected with lambda gt11 containing the HuIFN γ insert. The minilysates and a commercial source β -galactosidase are fractionated by SDS-PAGE, and the bands transferred to nitrocellulose filters
10 for Western blotting (Sambrook, *et al.*; Ausubel, *et al.*).

Summarizing the expected results, serum from control (Sj26) rabbits is immunoreactive with each of the Sj26 and Sj26 fused protein antigens. Serum from the animal immunized with Sj26/HuIFN γ fused protein is reactive with all Sj-26 and beta-gal fusion proteins containing HuIFN γ coding sequences, indicating the presence of specific immunoreaction with the HuIFN γ
15 antigen. None of the sera are expected to be immunoreactive with beta-galactosidase.

Anti-HuIFN γ antibody present in the sera from the animal immunized with the Sj26/HuIFN γ is purified by affinity chromatography (using immobilized recombinantly produced HuIFN γ as ligand, essentially as described above in Example 12 for the anti-beta-galactosidase antibody).
20

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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15 Methods of Use

(iii) NUMBER OF SEQUENCES: 34

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
35 (B) FILING DATE: 10-MAY-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/438,753
(B) FILING DATE: 10-MAY-1995

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(2) INFORMATION FOR SEQ ID NO:1:

50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 516 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
55 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

60 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Ovis aries
65 (B) STRAIN: Domestic
(D) DEVELOPMENTAL STAGE: Blastula (blastocyst)
(F) TISSUE TYPE: Trophectoderm

(G) CELL TYPE: Mononuclear trophectoderm cells

(vii) IMMEDIATE SOURCE:

(B) CLONE: oTP-1a

(viii) POSITION IN GENOME:

(C) UNITS: bp

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..516

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ott, Troy L

Van Heeke, Gino

Johnson, Howard M

Bazer, Fuller W

(B) TITLE: Cloning and Expression in *Saccharomyces cerevisiae* of a Synthetic Gene for the Type I Trophoblast Interferon Ovine Trophoblast Protein-1: Purification and Antiviral Activity

(C) JOURNAL: J. Interferon Res.

(D) VOLUME: 11

(F) PAGES: 357-364

(G) DATE: 1991

(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 516

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

30	TGC TAC CTG TCG CGA AAA CTG ATG CTG GAC GCT CGA GAA AAT TTA AAA Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys 1 5 10 15	48
35	CTG CTG GAC CGT ATG AAT CGA TTG TCT CCG CAC AGC TGC CTG CAA GAC Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp 20 25 30	96
40	CGG AAA GAC TTC GGT CTG CCG CAG GAA ATG GTT GAA GGT GAC CAA CTG Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu 35 40 45	144
45	CAA AAA GAC CAA GCT TTC CCG GTA CTG TAT GAA ATG CTG CAG CAG TCT Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser 50 55 60	192
50	TTC AAC CTG TTC TAC ACT GAA CAT TCT TCG GCC GCT TGG GAC ACT ACT Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr 65 70 75 80	240
55	CTT CTA GAA CAA CTG TGC ACT GGT CTG CAA CAG CAA CTG GAC CAT CTG Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu 85 90 95	288
60	GAC ACT TGC CGT GGC CAG GTT ATG GGT GAA GAA GAC TCT GAA CTG GGT Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly 100 105 110	336
65	AAC ATG GAT CCG ATC GTT ACT GTT AAA AAA TAT TTC CAG GGT ATC TAC Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr 115 120 125	384
	GAC TAC CTG CAG GAA AAA GGT TAC TCT GAC TGC GCT TGG GAA ATC GTA Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val 130 135 140	432
	CGC GTT GAA ATG ATG CGG GCC CTG ACT GTG TCG ACT ACT CTG CAA AAA Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys 145 150 155 160	480

CGG TTA ACT AAA ATG GGT GGT GAC CTG AAT TCT CCG
 Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
 165 170

516

5

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: amino acid sequence of a mature
 OvIFNtau protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
 1 5 10 15

Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
 20 25 30

Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu
 35 40 45

Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
 50 55 60

Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65 70 75 80

Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Leu Asp His Leu
 85 90 95

Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly
 100 105 110

Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr
 115 120 125

Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
 130 135 140

Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys
 145 150 155 160

Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
 165 170

55

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 588 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO

60

65

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

5 (C) INDIVIDUAL ISOLATE: HuIFNtau1 Human Interferon Tau coding
sequence with a leader sequence.

(ix) FEATURE:

(A) NAME/KEY: CDS

10

(B) LOCATION: 1..585

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

15	ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC TAC Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr	48
	1 5 10 15	
20	GGC CCA GGA GGA TCC CTG GGT TGT GAC CTG TCT CAG AAC CAC GTG CTG Gly Pro Gly Gly Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu	96
	20 25 30	
25	GTT GGC AGG AAG AAC CTC AGG CTC CTG GAC GAA ATG AGG AGA CTC TCC Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser	144
	35 40 45	
30	CCT CGC TTT TGT CTG CAG GAC AGA AAA GAC TTC GCT TTA CCC CAG GAA Pro Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu	192
	50 55 60	
35	ATG GTG GAG GGC GGC CAG CTC CAG GAG GCC CAG GCC ATC TCT GTG CTC Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu	240
	65 70 75 80	
40	CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA GAG CAC TCC His Glu Met Leu Gln Ser Phe Asn Leu Phe His Thr Glu His Ser	288
	85 90 95	
45	TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC ACT GGA CTC Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu	336
	100 105 110	
50	CAT CAG CAG CTG GAC AAC CTG GAT GCC TGC CTG GGG CAG GTG ATG GGA His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly	384
	115 120 125	
55	GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG GCT CTG AAG Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys	432
	130 135 140	
60	AGG TAC TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAC AGC Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser	480
	145 150 155 160	
65	GAC TGC GCC TGG GAA ACC GTC AGA CTG GAA ATC ATG AGA TCC TTC TCT Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser	528
	165 170 175	
	TCA TTA ATC AGC TTG CAA GAA AGG TTA AGA ATG ATG GAT GGA GAC CTG Ser Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu	576
	180 185 190	
	AGC TCA CCT TGA Ser Ser Pro	588
	195	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

5

- (A) LENGTH: 195 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
 of SEQ ID NO:3 (HuIFNtau1).

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
 1 5 10 15
 Gly Pro Gly Gly Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu
 20 20 25 30
 Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser
 25 35 40 45
 Pro Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu
 50 55 60
 Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
 30 65 70 75 80
 His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
 85 90 95
 Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu
 35 100 105 110
 His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
 40 115 120 125
 Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
 130 135 140
 Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
 45 145 150 155 160
 Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser
 165 170 175
 Ser Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu
 50 180 185 190
 Ser Ser Pro
 195

55

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

60

- (A) LENGTH: 25 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

65

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: 25-mer synthetic oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

5

CCTGTCTGCA GGACAGAAAA GACTT

25

(2) INFORMATION FOR SEQ ID NO:6:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(vi) ORIGINAL SOURCE:

20

(C) INDIVIDUAL ISOLATE: 25-mer synthetic oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

25

TCTGAATTCT GACGATTTCC CAGGC

25

(2) INFORMATION FOR SEQ ID NO:7:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 299 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

40

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HuIFNtau6

45

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2..298

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

C CAG GAG ATG GTG GAG GGC GGC CAG CTC CAG GAG GCC CAG GCC ATC
Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile
1 5 10 15

46

55

TCT GTG CTC CAC AAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA
Ser Val Leu His Lys Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr
20 25 30

94

60

GAG CGC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC
Glu Arg Ser Ser Ala Ala Trp Asp Thr Leu Leu Glu Gln Leu Arg
35 40 45

142

65

ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAC GCC TGC CTG GGG CAG
Thr Gly Leu His Gln Gln Leu Asp Leu Asp Ala Cys Leu Gly Gln
50 55 60

190

[illegible]

(2) INFORMATION FOR SEQ ID NO:8:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

25 (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
of SEQ ID NO:7 (HuIFNtau6).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

[illegible]

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HuIFNtau7

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2..286

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

C CAG GAG ATG GTG GAG GTC AGC CAG TTC CAG GAG GCC CAG GCC ATT 46
 Gln Glu Met Val Glu Val Ser Gln Phe Gln Glu Ala Gln Ala Ile
 1 5 10 15

TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC AAA 94
 Ser Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Lys
 20 25 30

GAG CGC TCC TCT GCT GCC TGG GAC ACT ACC CTC CTG GAG CAG CTC CTC 142
 Glu Arg Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Leu
 35 40 45

ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGT CTG GGG CAG 190
 Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln
 50 55 60

TTG ACT GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG 238
 Leu Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu
 65 70 75

GCC GTG AAG AGC TAC TTC CAG GGC ATC CAT GTC TAC CTG CAA GAG AAG 286
 Ala Val Lys Ser Tyr Phe Gln Gly Ile His Val Tyr Leu Gln Glu Lys
 80 85 90 95

GG 288

35 (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 95 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

45

- (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
 of SEQ ID NO:9 (HuIFNtau7).

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln Glu Met Val Glu Val Ser Gln Phe Gln Glu Ala Gln Ala Ile Ser
 1 5 10 15

Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Lys Glu
 20 25 30

Arg Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Leu Thr
 35 40 45

Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Leu
 50 55 60

Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
 65 70 75 80

Val Lys Ser Tyr Phe Gln Gly Ile His Val Tyr Leu Gln Glu Lys
 85 90 95

(2) INFORMATION FOR SEQ ID NO:11:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 307 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- 15 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: HuIFNtau4
- 20 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2..307

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

25 C CAG GAG ATG GTG GAG GGT GGC CAG CTC CAG GAG GCC CAG GCC ATC 46
 Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile
 1 5 10 15

30 TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA 94
 Ser Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr
 20 25 30

GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC 142
 35 Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg
 35 40 45

ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGC CTG GGG CAG 190
 40 Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln
 50 55 60

GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG 238
 Val Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu
 65 70 75

45 GCC ATG AAG ACG TAT TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG 286
 Ala Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
 80 85 90 95

50 GGA TAT AGT GAC TGC GCC TGG 307
 Gly Tyr Ser Asp Cys Ala Trp
 100

55 (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 60 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 65 (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
 of SEQ ID NO:11 (HuIFNtau4).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

5 Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser
 1 5 10 15
 Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu
 20 25 30
 10 His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr
 35 40 45
 Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val
 50 55 60
 15 Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
 65 70 75 80
 Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly
 85 90 95
 20 Tyr Ser Asp Cys Ala Trp
 100

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 294 base pairs
 (B) TYPE: nucleic acid
 30 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA to mRNA
 35 (iii) HYPOTHETICAL: NO
 (iv) ANTI-SENSE: NO
 (vi) ORIGINAL SOURCE:
 40 (C) INDIVIDUAL ISOLATE: HuIFNtau5
 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2..292

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

C CAG GAG ATG GTG GAG GGT GGC CAG CTC CAG GAG GCC CAG GCC ATC 46
 50 Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile 15
 TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA 94
 Ser Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr 20 25 30
 55 GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC 142
 Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg 35 40 45
 60 ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG GAT GCC TGC CTG GGG CAG 190
 Thr Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln 50 55 60
 65 GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG 238
 Val Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu 65 70 75

GCC ATG AAG ACG TAT TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG 286
 Ala Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
 80 85 90 95

5 GGA TAT AG 294
 Gly Tyr

(2) INFORMATION FOR SEQ ID NO:14:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

20

(C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence
 of SEQ ID NO:13 (HuIFNtau5).

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

25 Gln Glu Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser 15
 1 5 10
 Val Leu His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu
 20 25 30
 30 His Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr
 35 40 45
 Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val
 50 55 60
 35 Thr Gly Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala
 65 70 75 80
 40 Met Lys Thr Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly
 85 90 95
 Tyr

45 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 515 base pairs

50

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

55

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

60

(C) INDIVIDUAL ISOLATE: HuIFNtau2

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..513

65

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 115-117

(D) OTHER INFORMATION: /note= "to allow expression of the encoded protein this site can be modified to encode an amino acid, e.g., Gln"

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

	GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGG AAG AAC CTC AGG CTC	48
	Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg Leu	
10	1 5 10 15	
	CTG GAC CAA ATG AGG AGA CTC TCC CCT CGC TTT TGT CTG CAG GAC AGA	96
	Leu Asp Gln Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp Arg	
	20 25 30	
15	AAA GAC TTC GCT TTA CCC TAG GAA ATG GTG GAG GGC GGC CAG CTC CAG	144
	Lys Asp Phe Ala Leu Pro Glu Met Val Glu Gly Gln Leu Gln	
	35 40 45	
20	GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC	192
	Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser Phe	
	50 55 60	
	AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC	240
25	Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu	
	65 70 75 80	
	CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAC AAC CTG GAT	288
	Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu Asp	
30	85 90 95	
	GCC TGC CTG GGG CAG GTG ATG GGA GAG GAA GAC TCT GCC CTG GGA AGG	336
	Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly Arg	
	100 105 110	
35	ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG GGC ATC CAT GTC	384
	Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His Val	
	115 120 125	
40	TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC AGA	432
	Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val Arg	
	130 135 140	
	GTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA AGG	480
45	Val Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu Arg	
	145 150 155 160	
	TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT TG	515
50	Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro	
	165 170	

(2) INFORMATION FOR SEQ ID NO:16:

55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 171 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence of SEQ ID NO:15 (HuIFNtau2).

65

(ix) FEATURE:

- (A) NAME/KEY: Modified-site

(B) LOCATION: 39

(D) OTHER INFORMATION: /note= "where Xaa a selected amino acid, for example, Gln"

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

      Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg Leu
      1          5          10          15
10    Leu Asp Gln Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp Arg
      20          25          30
      Lys Asp Phe Ala Leu Pro Xaa Glu Met Val Glu Gly Gly Gln Leu Gln
      35          40          45
15    Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser Phe
      50          55          60
20    Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr Leu
      65          70          75
      Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu Asp
      85          90          95
25    Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly Arg
      100          105          110
      Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His Val
      115          120          125
30    Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val Arg
      130          135          140
      Val Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu Arg
      145          150          155          160
35    Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
      165          170

```

40

(2) INFORMATION FOR SEQ ID NO:17:

```

      (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 587 base pairs
45    (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)
50    (iii) HYPOTHETICAL: NO
      (iv) ANTI-SENSE: NO

      (vi) ORIGINAL SOURCE:
55    (C) INDIVIDUAL ISOLATE: HuIFNtau3, with leader seq.

      (ix) FEATURE:
      (A) NAME/KEY: CDS
60    (B) LOCATION: 1..585

```


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

5	ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC TAC Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr	48
	1 5 10 15	
10	GGC CCG GGA GGA TCC CTG CGG TGT GAC CTG TCT CAG AAC CAC GTG CTG Gly Pro Gly Ser Leu Arg Cys Asp Leu Ser Gln Asn His Val Leu	96
	20 25 30	
15	GTT GGC AGC CAG AAC CTC AGG CTC CTG GGC CAA ATG AGG AGA CTC TCC Val Gly Ser Gln Asn Leu Arg Leu Leu Gly Gln Met Arg Arg Leu Ser	144
	35 40 45	
20	CTT CGC TTC TGT CTG CAG GAC AGA AAA GAC TTC GCT TTC CCC CAG GAG Leu Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Phe Pro Gln Glu	192
	50 55 60	
25	ATG GTG GAG GGT GGC CAG CTC CAG GAG GCC CAG GCC ATC TCT GTG CTC Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu	240
	65 70 75 80	
30	CAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA GAG CAC TCC His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser	288
	85 90 95	
35	TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC ACT GGA CTC Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu	336
	100 105 110	
40	CAT CAG CAG CTG GAT GAC CTG GAT GCC TGC CTG GGG CAG GTG ACG GGA His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val Thr Gly	384
	115 120 125	
45	GAG GAA GAC TCT GCC CTG GGA AGA ACG GGC CCC ACC CTG GCC ATG AAG Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Met Lys	432
	130 135 140	
50	AGG TAT TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAT AGT Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser	480
	145 150 155 160	
55	GAC TGC GCC TGG GAA ATT GTC AGA CTG GAA ATC ATG AGA TCC TTG TCT Asp Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg Ser Leu Ser	528
	165 170 175	
60	TCA TCA ACC AGC TTG CAC AAA AGG TTA AGA ATG ATG GAT GGA GAC CTG Ser Ser Thr Ser Leu His Lys Arg Leu Arg Met Met Asp Gly Asp Leu	576
	180 185 190	
65	AGC TCA CCT TG Ser Ser Pro	587
	195	

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 195 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
- (C) INDIVIDUAL ISOLATE: predicted amino acid coding sequence of SEQ ID NO:17 (HuIFNtau3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

5 Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
 1 5 10 15
 Gly Pro Gly Gly Ser Leu Arg Cys Asp Leu Ser Gln Asn His Val Leu
 20 25 30
 10 Val Gly Ser Gln Asn Leu Arg Leu Leu Gly Gln Met Arg Arg Leu Ser
 35 40 45
 Leu Arg Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Phe Pro Gln Glu
 50 55 60
 15 Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
 65 70 75 80
 His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
 85 90 95
 20 Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu
 100 105 110
 25 His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val Thr Gly
 115 120 125
 Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Met Lys
 130 135 140
 30 Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
 145 150 155 160
 Asp Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg Ser Leu Ser
 165 170 175
 35 Ser Ser Thr Ser Leu His Lys Arg Leu Arg Met Met Asp Gly Asp Leu
 180 185 190
 40 Ser Ser Pro
 195

(2) INFORMATION FOR SEQ ID NO:19:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 518 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 50 (ii) MOLECULE TYPE: DNA (genomic)
 (iii) HYPOTHETICAL: NO
 55 (iv) ANTI-SENSE: NO
 (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: HuIFNtau3, mature, no leader sequence
 60 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..516

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5 TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGC CAG AAC CTC AGG 48
 Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
 1 5 10 15
 CTC CTG GGC CAA ATG AGG AGA CTC TCC CTT CGC TTC TGT CTG CAG GAC 96
 Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
 20 25 30
 10 AGA AAA GAC TTC GCT TTC CCC CAG GAG ATG GTG GAG GGT GGC CAG CTC 144
 Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gln Leu
 35 40 45
 15 CAG GAG GCC CAG GCC ATC TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC 192
 Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
 50 55 60
 20 TTC AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC 240
 Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65 70 75 80
 CTC CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG 288
 Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asp Leu
 25 85 90 95
 GAT GCC TGC CTG GGG CAG GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA 336
 Asp Ala Cys Leu Gly Gln Val Thr Gly Glu Glu Asp Ser Ala Leu Gly
 100 105 110
 30 AGA ACG GGC CCC ACC CTG GCC ATG AAG AGG TAT TTC CAG GGC ATC CAT 384
 Arg Thr Gly Pro Thr Leu Ala Met Lys Arg Tyr Phe Gln Gly Ile His
 115 120 125
 35 GTC TAC CTG AAA GAG AAG GGA TAT AGT GAC TGC GCC TGG GAA ATT GTC 432
 Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
 130 135 140
 40 AGA CTG GAA ATC ATG AGA TCC TTG TCT TCA TCA ACC AGC TTG CAC AAA 480
 Arg Leu Glu Ile Met Arg Ser Leu Ser Ser Thr Ser Leu His Lys
 145 150 155 160
 45 AGG TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT TG 518
 Arg Leu Arg Met Met Asp Gly Asp Leu Ser Pro
 165 170

(2) INFORMATION FOR SEQ ID NO:20:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

60 Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
 1 5 10 15
 Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
 20 25 30
 65 Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu
 35 40 45

25 (2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 37 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35 (vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
1-37 of SEQ ID NO:20

```

40          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
      Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
        1          5          10          15
45      Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
          20          25          30
      Arg Lys Asp Phe Ala
          35

```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
 34-64 of SEQ ID NO:20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

5 Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu Gln
 1 5 10 15
 Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
 20 25 30

10 (2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 15
 (ii) MOLECULE TYPE: protein
 (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
 20 62-92 of SEQ ID NO:20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

25 Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp
 1 5 10 15
 Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln
 30 20 25 30

(2) INFORMATION FOR SEQ ID NO:24:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 40 (ii) MOLECULE TYPE: protein
 (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
 45 90-122 of SEQ ID NO:20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

50 His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln Val Thr Gly
 1 5 10 15
 Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Met Lys
 20 25 30
 55 Arg

(2) INFORMATION FOR SEQ ID NO:25:

60 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 65 (ii) MOLECULE TYPE: protein
 (vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
119-150 of SEQ ID NO:20

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Met Lys Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
1 5 10 15
10 Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg
20 25 30

15 (2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

25 (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
139-172 of SEQ ID NO:20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

30 Cys Ala Trp Glu Ile Val Arg Leu Glu Ile Met Arg Ser Leu Ser Ser
1 5 10 15
Ser Thr Ser Leu His Lys Arg Leu Arg Met Met Asp Gly Asp Leu Ser
20 25 30
35 Ser Pro

40 (2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

50 (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
1-23 of SEQ ID NO:18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

55 Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
1 5 10 15
Gly Pro Gly Gly Ser Leu Arg
20

60

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

65

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
1-23 of SEQ ID NO:4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
1 5 10 15
Gly Pro Gly Gly Ser Leu Gly
20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 519 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HuIFNtaul genomic-derived
DNA coding sequence, without leader seq.

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..516

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGG AAG AAC CTC AGG 48
Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
1 5 10 15
CTC CTG GAC GAA ATG AGG AGA CTC TCC CCT CGC TTT TGT CTG CAG GAC 96
Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
20 25 30
AGA AAA GAC TTC GCT TTA CCC CAG GAA ATG GTG GAG GGC GGC CAG CTC 144
Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
35 40 45
CAG GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC 192
Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
50 55 60
TTC AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC 240
Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
65 70 75 80
CTC CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAC AAC CTG 288
Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
85 90 95
GAT GCC TGC CTG GGG CAG GTG ATG GGA GAG GAA GAC TCT GCC CTG GGA 336
Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
100 105 110

52

AGG ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG GGC ATC CAT 384
 Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
 115 120 125
 5 GTC TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC 432
 Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
 130 135 140
 10 AGA CTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA 480
 Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
 145 150 155 160
 15 AGG TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT TGA 519
 Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
 165 170

(2) INFORMATION FOR SEQ ID NO:30:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
 30 Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
 1 5 10 15
 Leu Leu Asp Glu Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
 20 25 30
 35 Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
 35 40 45
 Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
 50 55 60
 Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65 70 75 80
 45 Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
 85 90 95
 Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
 100 105 110
 50 Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
 115 120 125
 Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
 130 135 140
 55 Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
 145 150 155 160
 60 Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
 165 170

65 (2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

53

(A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HuIFNtau Group I core
 sequence (HuIFNtauGRI).

10

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 20

(D) OTHER INFORMATION: /note= "where Xaa is Glu or Gln"

15

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 146

(D) OTHER INFORMATION: /note= "where Xaa is Leu or Val"

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
 1 5 10 15

25

Leu Leu Asp Xaa Met Arg Arg Leu Ser Pro Arg Phe Cys Leu Gln Asp
 20 25 30

30

Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
 35 40 45

Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
 50 55 60

35

Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65 70 75 80

Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
 85 90 95

40

Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
 100 105 110

45

Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
 115 120 125

Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
 130 135 140

50

Arg Xaa Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
 145 150 155 160

Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
 165 170

55

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 172 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: protein

65

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HuIFNtau Group II core

sequence (HuIFNtauGRII).

(ix) FEATURE:

5

(A) NAME/KEY: Modified-site

(B) LOCATION: 122

(D) OTHER INFORMATION: /note= "where Xaa is Arg or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

10 Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg
 1 5 10 15
 Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp
 20 25 30
 15 Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu
 35 40 45
 20 Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
 50 55 60
 Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
 65 70 75 80
 25 Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asp Leu
 85 90 95
 Asp Ala Cys Leu Gly Gln Val Thr Gly Glu Glu Asp Ser Ala Leu Gly
 100 105 110
 30 Arg Thr Gly Pro Thr Leu Ala Met Lys Xaa Tyr Phe Gln Gly Ile His
 115 120 125
 35 Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
 130 135 140
 Arg Leu Glu Ile Met Arg Ser Leu Ser Ser Ser Thr Ser Leu His Lys
 145 150 155 160
 40 Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
 165 170

45 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 99 amino acids

50 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

55 (C) INDIVIDUAL ISOLATE: HuIFNtau Group III core
 sequence (HuIFNtauGRIII).

(ix) FEATURE:

(A) NAME/KEY: Modified-site

60 (B) LOCATION: 7

(D) OTHER INFORMATION: /note= "where Xaa is Gly or Ser"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

65 (B) LOCATION: 9

(D) OTHER INFORMATION: /note= "where Xaa is Leu or Phe"

- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 20
 (D) OTHER IFNORMATION: /note= "where Xaa is Lys or Glu"
- 5
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER IFNORMATION: /note= "where Xaa is Thr or Lys"
- 10
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 47
 (D) OTHER IFNORMATION: /note= "where Xaa is Arg or Leu"
- 15
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 64
 (D) OTHER IFNORMATION: /note= "where Xaa is Val or Leu"
- 20
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 90
 (D) OTHER IFNORMATION: /note= "where Xaa is Ile or Val"
- 25
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Glu | Met | Val | Glu | Gly | Xaa | Gln | Xaa | Gln | Glu | Ala | Gln | Ala | Ile | Ser |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Val | Leu | His | Xaa | Met | Leu | Gln | Gln | Ser | Phe | Asn | Leu | Phe | His | Xaa | Glu |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Arg | Ser | Ser | Ala | Ala | Trp | Asp | Thr | Thr | Leu | Leu | Glu | Gln | Leu | Xaa | Thr |
| | | | 35 | | | | 40 | | | | | 45 | | | |
| Gly | Leu | His | Gln | Gln | Leu | Asp | Asp | Leu | Asp | Ala | Cys | Leu | Gly | Gln | Xaa |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Thr | Gly | Glu | Glu | Asp | Ser | Ala | Leu | Gly | Arg | Thr | Gly | Pro | Thr | Leu | Ala |
| | 65 | | | | 70 | | | | 75 | | | | | 80 | |
| Val | Lys | Ser | Tyr | Phe | Gln | Gly | Ile | His | Xaa | Tyr | Leu | Gln | Glu | Lys | Gly |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Tyr | Ser | Asp | | | | | | | | | | | | | |
- 45
- (2) IFNORMATION FOR SEQ ID NO:34:
- 50
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- 55
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 (C) INDIVIDUAL ISOLATE: HuIFNtau core
 sequence (HuIFNtauCS).
- 60
- (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 12
 (D) OTHER IFNORMATION: /note= "where Xaa is Arg or Ser"
- 65

- 5 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER IFNORMATION: /note= "where Xaa is Lys or Gln"
- 10 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER IFNORMATION: /note= "where Xaa is Asp or Gly"
- 15 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 20
(D) OTHER IFNORMATION: /note= "where Xaa is Glu or Gln"
- 20 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 26
(D) OTHER IFNORMATION: /note= "where Xaa is Pro or Leu"
- 25 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 38
(D) OTHER IFNORMATION: /note= "where Xaa is Leu or Phe"
- 30 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 45
(D) OTHER IFNORMATION: /note= "where Xaa is Gly or Val"
- 35 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 46
(D) OTHER IFNORMATION: /note= "where Xaa is Gly or Ser"
- 40 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 48
(D) OTHER IFNORMATION: /note= "where Xaa is Leu or Phe"
- 45 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 59
(D) OTHER IFNORMATION: /note= "where Xaa is Lys or Glu"
- 50 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 70
(D) OTHER IFNORMATION: /note= "where Xaa is Thr or Lys"
- 55 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 72
(D) OTHER IFNORMATION: /note= "where Xaa is Arg or His"
- 60 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 86
(D) OTHER IFNORMATION: /note= "where Xaa is Arg or Leu"
- 65 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 95
(D) OTHER IFNORMATION: /note= "where Xaa is Asn or Asp"
- (ix) FEATURE:
(A) NAME/KEY: Modified-site

- (B) LOCATION: 103
(D) OTHER INFORMATION: /note= "where Xaa is Val or Leu"
- 5 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 104
(D) OTHER INFORMATION: /note= "where Xaa is Met or Thr"
- 10 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 120
(D) OTHER INFORMATION: /note= "where Xaa is Val, Leu or Met"
- 15 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 122
(D) OTHER INFORMATION: /note= "where Xaa is Arg, Ser or Thr"
- 20 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 129
(D) OTHER INFORMATION: /note= "where Xaa is Ile or Val"
- 25 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 132
(D) OTHER INFORMATION: /note= "where Xaa is Lys or Gln"
- 30 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 143
(D) OTHER INFORMATION: /note= "where Xaa is Ile or Thr"
- 35 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 146
(D) OTHER INFORMATION: /note= "where Xaa is Leu or Val"
- 40 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 152
(D) OTHER INFORMATION: /note= "where Xaa is Leu or Phe"
- 45 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 155
(D) OTHER INFORMATION: /note= "where Xaa is Leu or Ser"
- 50 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 156
(D) OTHER INFORMATION: /note= "where Xaa is Ile or Thr"
- 55 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 159
(D) OTHER INFORMATION: /note= "where Xaa is Gln or His"
- 60 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 160
(D) OTHER INFORMATION: /note= "where Xaa is Glu or Lys"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

	Cys	Asp	Leu	Ser	Gln	Asn	His	Val	Leu	Val	Gly	Xaa	Xaa	Asn	Leu	Arg	
	1				5					10					15		
5	Leu	Leu	Xaa	Xaa	Met	Arg	Arg	Leu	Ser	Xaa	Arg	Phe	Cys	Leu	Gln	Asp	
				20					25					30			
	Arg	Lys	Asp	Phe	Ala	Xaa	Pro	Gln	Glu	Met	Val	Glu	Xaa	Xaa	Gln	Xaa	
10			35					40					45				
	Gln	Glu	Ala	Gln	Ala	Ile	Ser	Val	Leu	His	Xaa	Met	Leu	Gln	Gln	Ser	
		50					55					60					
15	Phe	Asn	Leu	Phe	His	Xaa	Glu	Xaa	Ser	Ser	Ala	Ala	Trp	Asp	Thr	Thr	
	65					70					75					80	
	Leu	Leu	Glu	Gln	Leu	Xaa	Thr	Gly	Leu	His	Gln	Gln	Leu	Asp	Xaa	Leu	
					85				90					95			
20	Asp	Ala	Cys	Leu	Gly	Gln	Xaa	Xaa	Gly	Glu	Glu	Asp	Ser	Ala	Leu	Gly	
				100					105					110			
	Arg	Thr	Gly	Pro	Thr	Leu	Ala	Xaa	Lys	Xaa	Tyr	Phe	Gln	Gly	Ile	His	
25			115					120					125				
	Xaa	Tyr	Leu	Xaa	Glu	Lys	Gly	Tyr	Ser	Asp	Cys	Ala	Trp	Glu	Xaa	Val	
		130					135					140					
30	Arg	Xaa	Glu	Ile	Met	Arg	Ser	Xaa	Ser	Ser	Xaa	Xaa	Ser	Leu	Xaa	Xaa	
	145					150					155					160	
	Arg	Leu	Arg	Met	Met	Asp	Gly	Asp	Leu	Ser	Ser	Pro					
35					165					170							

IT IS CLAIMED:

1. An isolated human interferon- γ protein, wherein said protein contains a mature interferon- γ polypeptide having a sequence derived from the sequence presented as SEQ ID NO:34.
5
2. An isolated human interferon- γ protein of claim 1, wherein said protein contains a mature interferon- γ polypeptide having a sequence derived from the sequence presented as SEQ ID NO:31.
10
3. An isolated human interferon- γ protein of claim 2, wherein said mature polypeptide has the sequence presented as SEQ ID NO:30.
4. An isolated human interferon- γ protein of claim 3, where said mature polypeptide is encoded by the sequence presented as SEQ ID NO:29.
15
5. An isolated human interferon- γ protein of claim 2, wherein said mature polypeptide has the sequence presented as SEQ ID NO:16.
- 20 6. An isolated human interferon- γ protein of claim 5, where said mature polypeptide is encoded by the sequence presented as SEQ ID NO:15.
7. An isolated human interferon- γ protein of claim 1, wherein said protein contains a mature interferon- γ polypeptide having a sequence derived from the sequence presented as SEQ ID NO:32.
25
8. An isolated human interferon- γ protein of claim 7, wherein said mature polypeptide has the sequence presented as SEQ ID NO:20.
- 30 9. An isolated human interferon- γ protein of claim 8, where said mature polypeptide is encoded by the sequence presented as SEQ ID NO:19.
10. An isolated human interferon- γ protein of claim 7, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:12.

11. An isolated human interferon- τ protein of claim 10, where said polypeptide is encoded by the sequence presented as SEQ ID NO:11.

12. An isolated human interferon- τ protein of claim 7, wherein said protein contains
5 a polypeptide having the sequence presented as SEQ ID NO:14.

13. An isolated human interferon- τ protein of claim 12, where said polypeptide is encoded by the sequence presented as SEQ ID NO:13.

10 14. An isolated human interferon- τ protein of claim 1, wherein said protein contains a mature interferon- τ polypeptide having a sequence derived from the sequence presented as SEQ ID NO:33.

15 15. An isolated human interferon- τ protein of claim 14, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:8.

16. An isolated human interferon- τ protein of claim 15, where said polypeptide is encoded by the sequence presented as SEQ ID NO:7.

20 17. An isolated human interferon- τ protein of claim 14, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:10.

18. An isolated human interferon- τ protein of claim 17, where said polypeptide is encoded by the sequence presented as SEQ ID NO:9.

25 19. An isolated human interferon- τ protein, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:8.

20. An isolated human interferon- τ protein, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:10.
30

21. An isolated human interferon- τ protein, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:12.

22. An isolated human interferon- τ protein, wherein said protein contains a polypeptide having the sequence presented as SEQ ID NO:14.

23. An isolated human interferon- τ protein of any of claims 1 to 22, where said polypeptide further includes a leader sequence.

24. An isolated human interferon- τ protein of any of claims 1 to 23, where said protein is recombinantly produced.

25. An expression vector comprising

(a) a nucleic acid containing an open reading frame that encodes a human interferon- τ of any of claims 1 to 22; and

(b) regulatory sequences effective to express said open reading frame in a host cell.

15

26. A method of recombinantly producing human interferon- τ , comprising introducing into suitable host cells, an expression vector of claim 25, where the vector contains an open reading frame (ORF) having a polynucleotide sequence which encodes a human interferon- τ polypeptide, where the vector is designed to express the ORF in said host,

20

and

culturing said host under conditions resulting in the expression of the ORF sequence.

27. A method of inhibiting tumor cell growth, comprising contacting the cells with a human τ -interferon of any of claims 1 to 22, at a concentration effective to inhibit growth of the tumor cells.

25

28. A method of claim 27, wherein said cells are human carcinoma cells, human leukemia cells, human T-lymphoma cells, and human melanoma cells.

30

29. A method of claim 27, wherein said cells are steroid-sensitive tumor cells.

30. A method of claim 29, wherein said cells are mammary tumor cells.

31. A method of inhibiting viral replication, comprising

contacting cells infected with a virus with a human γ -interferon of any of claims 1 to 15, at a concentration effective to inhibit viral replication within said cells.

32. A method of claim 31, where said virus is an RNA virus.

5

33. A method of claim 32, where said virus is human immunodeficiency virus or hepatitis c virus.

34. A method of claim 31, where said virus is a DNA virus.

10

35. A method of claim 34, where said virus is hepatitis B virus.

36. A method of enhancing fertility in a female mammal, comprising administering to said mammal an effective mammalian fertility enhancing amount of a human interferon- γ of any of claims 1 to 22, in a pharmaceutically acceptable carrier.

15

1
CTGAGATGGGATCAGAGAACCTACCTGAAGGTTCCCCCTGACCCCATCTCAGCCAGCCCAGCAGCAGCCGCATCTTCCCC 80

81
ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC TAT GGC CCA GGA GGA 140
S1 S20
Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr Gly Pro Gly Gly

141
TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC AGG GAG AAC CTC AAG CTC 200
S21 17
Ser Leu Gly Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys Leu

201
CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG CAG GAC AGA AAA GAC TTT GGT 260
18 37
Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp Arg Lys Asp Phe Gly

261
CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG AAG GAC CAG GCC TTC CCT GTG CTC 320
38 57
Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu Gln Lys Asp Gln Ala Phe Pro Val Leu

321
TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC TAC ACA GAG CAC TCC TCT GCT GCC TGG 380
58 77
Tyr Glu Met Leu Gln Gln Ser Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp

381
GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC 440
78 97
Asp Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu Asp

441
ACC TGC AGG GGT CAA GTG ATG GGA GAG GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT 500
98 117
Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly Asn Met Asp Pro Ile

501
GTG ACC GTG AAG AAG TAC TTC CAG GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC 560
118 137
Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr Asp Tyr Leu Gln Glu Lys Gly Tyr Ser

561
GAC TGC GCC TGG GAA ATC GTC AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC 620
138 157
Asp Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr

621
TTG CAA AAA AGG TTA ACA AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA TGACTCTTGCCGACTA 666
158 172
Leu Gln Lys Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro

AGATGCCACATCAGCCTCCTACACCCGCTGTGTTTCATTTCAGAAGACTCTGATTTCTGCTCCAGCCACCAAATTCATTG 764

AATTACTTTAGCTGATACTTTGTGTCAGTAGTAAAAAGCAAGTAGATATAAAAGTATTCAGCTGTAGGGGCATGAGTCCTGA 844

AATGATGCCTTCCCTGATGTTATCTGTTGCTGATTTATTTATACCTTCTAGCATTTAACATACTTAAATATTAGGAAAT 924

TTGTTAAGTTACATTTACATCTGTACATCATATTAATAATTTCTAAACAAAAA 972

Fig. 1

```

-23                                     -9
      met ala phe val leu ser leu leu met ala leu val leu val ser
oIFNt      cccc ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC
huIFNt1     cccc ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC

-8                                     -1 +1                                     11
tyr gly pro gly gly ser leu gly cys tyr leu ser arg lys leu met leu asp ala
TAT GGC CCA GGA GGA TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC
TAC GGC CCA GGA GGA TCC CTG GGT TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC
                                     asp                               gln asn his val val gly

12                                     30
arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
AGG GAG AAC CTC AAG CTC CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG
AGG AAG AAC CTC AGG CTC CTG GAC GAA ATG AGG AGA CTC TCC CCT CGC TTT TGT CTG
      lys          arg          glu          arg          arg phe

31                                     49
gln asp arg lys asp phe gly leu pro gln glu met val glu gly arg gln leu gln
CAG GAC AGA AAA GAC TTT GGT CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG
CAG GAC AGA AAA GAC TTC GCT TTA CCC CAG GAA ATG GTG GAG GGC GGC CAG CTC CAG
                                     ala                               gly

50                                     68
lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
AAG GAC CAG GCC TTC CCT GTG CTC TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
glu ala          ile ser          his

69                                     87
tyr thr glu his ser ser ala ala trp asp thr thr leu leu glu gln leu cys thr
TAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT
CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC GGC ACT
his                                     arg

88                                     106
gly leu gln gln gln leu asp his leu asp thr cys arg gly gln val met gly glu
GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC ACC TGC AGG GGT CAA GTG ATG GGA GAG
GGA CTC CAT CAG CAG CTG GAC AAC CTG GAT GCC TGC CTG GGG CAG GTG ATG GGA GAG
      his          asn          ala          leu

107                                     125
glu asp ser glu leu gly asn met asp pro ile val thr val lys lys tyr phe gln
GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT GTG ACC GTG AAG AAG TAC TTC CAG
GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG
      ala          arg thr gly          thr leu ala leu          arg

126                                     144
gly ile tyr asp tyr leu gln glu lys gly tyr ser asp cys ala trp glu ile val
GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ATC GTC
GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC
      his val          lys          thr

145                                     163
arg val glu met met arg ala leu thr val ser thr thr leu gln lys arg leu thr
AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC TTG CAA AAA AGG TTA ACA
AGA CTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA AGG TTA AGA
      leu          ile          ser phe ser ser leu ile ser          glu          arg

164                                     172
lys met gly gly asp leu asn ser pro end
AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA
ATG ATG GAT GGA GAC CTG AGC TCA CCT TGA
met      asp          ser

```

Fig. 2

```

                                -23                                     -9
                                Met ala phe val leu ser leu leu met ala leu val leu val ser
oINFt                          ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC
HuIFNt1                        c
HuIFNt2                        c
HuIFNt3                        c

-8                                -1 +1                                     11
tyr gly pro gly gly ser leu gly cys tyr leu ser arg lys leu met leu asp ala
TAT GGC CCA GGA GGA TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC
c                                     G g A C A G T G
c                                     (---)G g A C A G T G
c g c C G G g A C A G T G

12                                20                                     30
arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
AGG GAG AAC CTC AAG CTC CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG
A G GA GG GC TT
A G A GG GC TT
C C G G A GG T GC T

31                                40                                     49
gln asp arg lys asp phe gly leu pro gln glu met val glu gly asp gln leu gln
CAG GAC AGA AAA GAC TTT GGT CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG
c C t a a G
c C t a TAG a G
c C t C t G
HuIFNt4 ! t G
HuIFNt5 ! t G
HuIFNt6 ! G
HuIFNt7 ! T AG T

50                                60                                     68
lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
AAG GAC CAG GCC TTC CCT GTG CTC TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
G C A T C T
G C A T C T
G C A T C
G C A T C
G C A T C A
G C A T T C T

69                                80                                     87
tyr thr glu his ser ser ala ala try asp thr thr leu leu glu gln leu cys thr
TAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT
C C C C C C C
C A G CT

```

Fig. 3A

[illegible]

Fig. 3B

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                                -23                                     -9
oTP-1      Met ala phe val leu ser leu leu met ala leu val leu val ser
HuIFN $\alpha$ 1
HuIFN $\alpha$ 2
HuIFN $\alpha$ 3

-8                                -1 +1                                     11
tyr gly pro gly gly ser leu gly cys tyr leu ser arg lys leu met leu asp ala
                                asp                                gln asn his val val gly
                                (---)asp                           gln asn his val val gly
                                arg      asp                        gln asn his val val gly

12                                20                                     30
arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
    lys                arg                glu      arg                arg phe
    lys                arg                gln      arg                arg phe
ser gln                arg                gly gln      arg                leu arg phe

31                                40                                     49
gln asp arg lys asp phe gly leu pro gln glu met val glu gly asp gln leu gln
                                ala                                gly
                                ala                                gly
                                ala phe                            gly
HuIFN $\alpha$ 4                                !                                gly
HuIFN $\alpha$ 5                                !                                gly
HuIFN $\alpha$ 6                                !                                gly
HuIFN $\alpha$ 7                                !                                val ser      phe

50                                60                                     68
lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
glu ala                ile ser                his
glu ala                ile ser                his
glu ala                ile ser                his
glu ala                ile ser                his
glu ala                ile ser                his
glu ala                ile ser                his lys
glu ala                ile ser                his

69                                80                                     87
tyr thr glu his ser ser ala ala trp asp thr thr leu leu glu gln leu cys thr
his                                                                arg
his                                                                arg
his                                                                arg
his                                                                arg
his                                                                arg
his lys      arg                                                                arg
his lys      arg                                                                leu

```

Fig. 4A

[illegible]

Fig. 4B

INTERNATIONAL SEARCH REPORT

In' onal Application No

PCT/US 96/06911

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/20 C07K14/555 A61K38/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 10313 (UNIVERSITY OF FLORIDA) 11 May 1994 cited in the application see sequences n0 3, 4, 11 and 12 see page 8, line 12 - page 9 see claims; examples 5-7 ---	1,3, 23-36
A	JOURNAL OF BIOLOGICAL CHEMISTRY (MICROFILMS), vol. 269, no. 14, 8 April 1994, MD US, pages 10864-10868, XP002010875 A.E. WHALEY ET AL: "Identification and cellular localization of unique interferon mRNA from human placenta" see the whole document --- -/-	1,3, 31-33

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search

14 August 1996

Date of mailing of the international search report

28.08.96

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Le Cornec, N

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/06911

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE, vol. 330, 26 November 1987, LONDON GB, pages 377-379, XP002010876 K.IMAKAWA ET AL: "Interferon like sequence of ovine trophoblast protein secreted by embryonic trophectoderm" cited in the application see the whole document -----</p>	1

INTERNATIONAL SEARCH REPORT

national application No.

PCT/US 96/06911

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US96/06911

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claims 27-35 (as far as they do not concern in vivo methods) and claim 36 are related to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/06911

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9410313	11-05-94	AU-B- 5444994	24-05-94
		CN-A- 1090510	10-08-94
		EP-A- 0669981	06-09-95
		JP-T- 8505047	04-06-96

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